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Influence of water supply and fluctuations on yield and quality of lemon balm (*Melissa officinalis* L.)

Zámboriné Németh É., Radácsi P., Gosztola B., Rajhárt P., Szabó K.

Szent István University, Department of Medicinal and Aromatic Plants, Budapest, Hungary

***Corresponding author**: **zamborine.nemeth.eva@kertk.szie.hu**

Abstract

The response of lemon balm (*Melissa officinalis*) on drought stress was investigated in a pot experiment in 2015-16. In treatment S1, plants grew in 40% saturation of soil water capacity (SWC) for six weeks. In treatment S2, the same level of water supply was used for three weeks, while the control plants received irrigation up to 70% SWC throughout the experimental period. In the second year, after the stress period, a 3 week re-hydration phase was also carried out. Growth, morphology, biomass, active components and antioxidant capacity of the plants were measured. Six weeks growth at reduced soil water content (40% soil water capacity) resulted in a significant decrease of all morphological features of lemon balm as well as the fresh mass of shoots, as compared to the control (70% SWC). The effect of the shorter drought treatment (3 weeks) was less characteristic. After the three week recovery period no significant difference concerning number of branches, leaf length and fresh shoot mass was detectable among the plants. Thus, the retarding effect of even the 6 week drought stress (S1) was reversible in lemon balm. The changes in active components (volatile compounds, phenolics, rosmarinic acid) and antioxidant capacity did not show meaningful changes due to the treatments. After a three-week re-hydration period a significant increase was found in each parameter. It was established that a six week period of water shortage (water content of 40% SWC) could threaten the yield of lemon balm, although re-hydration by regular irrigation may induce significant recovery. The impact of water shortage on the quality of the drug was less critical.

Keywords: antioxidant capacity, biomass, drought stress, essential oil, regeneration, phenolics, rosmarinic acid, water potential.
Abbreviations: AAE_ascorbic acid equivalent; AC_antioxidant capacity; EO_essential oil; FRAP_ferric reducing ability of plasma; GAE_gallic acid equivalent; RA_rosmarinic acid; RWC_relative water content; SWC_soil water capacity; SPAD_equipment which measures chlorophyll content based on leaf colour intensity; TPC_total phenolic content.

Introduction

Assuring optimal water supply is a challenge in field crop production. In the cultivation of medicinal and aromatic plants we are facing even more difficulties. In the case of the majority of these species, scientific results on the effects of drought are still limited and often contradictory, which demonstrates that the question is complex. Although both yield and quality are important, the optimum of dry matter production does not necessarily coincide with that of the accumulation of secondary compounds (Penka, 1978, Bernáth és Németh, 2001).

Lemon balm (*Melissa officinalis* L.) is a perennial species known and cultivated all around the world. According to the Community Herbal Monograph (2013) the dry leaf (*Melissae folium*) is justified as a sleep aid, a relief of mild symptoms of mental stress, and effective against mild gastrointestinal complaints. Its extract has strong antioxidant capacity and is used also against *Herpes simplex* virus (Barnes et al., 200,; Rusaczonek et al., 2007). The most important biologically active components are the essential oil (EO) and the phenolics, among them flavonoids (mainly glycosides of luteolin) and rosmarinic acid (RA).

In practice, lemon balm is considered to be a plant that prefers good soil conditions, abundant nutrition and precipitation (Pank, 1991, Hoppe, 2013). However, only very little data is available documenting the effects of environmental factors including water supply. Ozturk et al. (2004) detected a significant loss of yield but an increase of EO accumulation if water deficit of the soil exceeded 25%. Shirzadi et al. (2010), however, did not find any significant change in the biomass production of lemon balm due to drought stress. Unfortunately, in these publications the water dosage was not precisely defined. Farahany et al. (2009) reported the highest EO content at the lowest water supply (20% field capacity) while the highest plant height and fresh mass was obtained at full water capacity of the soil.

Investigation in soilless conditions on accumulation of different active substances in lemon balm due to different water supply has only been carried out by Manukyan (2011), who found a significant increase in the amounts of EO, total phenolic content (TPC) and RA under severe drought conditions (250 hPa soil moisture). In our recent experiment in a climatic chamber, we compared lemon balm plants grown in sandy soil containing water up to 25, 40 and 70% of soil water capacity (SWC), respectively. It was found that poor water supply resulted in significantly reduced shoot mass production, while the applied treatments did not affect TPC and RA contents (Németh-Zámbori et al., 2016).

In 2015-16 we initiated a pot experiment to acquire more detailed, well established data on the drought behaviour of lemon balm. The goal of the trial was to answer the following questions: how does a six-week drought period – which might also occur under natural conditions – influence plant growth and biomass? Are these changes related to alterations in the concentration of active ingredients like essential oil

(EO), total phenolic content (TPC), rosmarinic acid content (RA) and antioxidant capacity (AC) of the drug? Are the changes reversible and if yes, to what extent could the plants recover?

Results

Effect of drought on physiological characteristics representing plant water status

Water potential – as anticipated – was the highest in plants subjected to a continuous stress situation (S2), (Table 1.). It is higher by 35% (in 2015) and 65% (in 2016) than that of the control (C). Treatment S1 exhibited medium values, showing that these plants could not reach the φ of the plants that had already been lacking water for a longer period. After the regeneration phase, when all plants were growing in soils of 70% SWC, water potential of the stressed plants showed lower values than immediately at the end of the drought period. As the water potential of plants reflects changing situations, it shows that these plants were able to drop their water potential when water was abundantly available and reach values similar to the control.

As presumed, relative water content (RWC) of the experimental plants decreases by decreasing the water level of the soil, although the difference reached a significant level only in the first year (Table 1.). In parallel with this, dry matter content of the plant samples was significantly higher in both stressed treatments and both years, as compared to the control.

During the re-hydration phase, RWC increases in S1 and S2 reaching the values of C with no significant difference. In parallel, due to the improved water supply, dry matter content decreases (water content increases) in the variants which had been stressed previously, however, the difference between them and the control plants still remained after 3 weeks.
In both stressed treatments (S1 and S2) higher SPAD chlorophyll values were detected than in the control plants (Table 1). The difference was significant for both treatments in 2016 and for S1 in 2015. After the three-week re-hydration period - contrary to the above mentioned parameters -SPAD chlorophyll content still did not change significantly. However, this seems obvious, because the leaves being investigated originated from the third internodes of the shoot tip, where the majority of leaves had still developed during the drought period.

Effect of drought on morphological characteristics and biomass

Concerning the evaluated morphological characteristics, the plants of continuous water shortage (S2) and those of the control treatment differed significantly at a high probability level in both years (Table 2.). Additionally, the biomass data showed a significant decline due to the water shortage compared to the plants grown in soil of 70% SWC. The only exception is the root mass in 2016, however, also in this case the root production of stressed plants reached only 74% of that of the controls.

The difference between S1 and the C was more variable. In 2015 for each trait (except leaf size), S1 was also significantly lower than the values of the control. Under the conditions of the second year, treatment S1 did not differ significantly from the control in the majority of characteristics except the leaf size.

Nevertheless, in harmony with our assumptions, the values of S1 were most frequently between the values of C and S2, except leaf size in 2015 and plant height in 2016. As a consequence of the drought treatments, the ratio between the mass of roots and that of the shoots showed the highest values in S2 in both years while it was lower by 64-86% and 79-88% in S1 and C, respectively.

During re-hydration we found that the plants which had been grown in severe drought conditions (S2) earlier started accelerated growth, and the measured parameters increased (Table 2.). Plant height rose significantly (by 37.6%), but three weeks were obviously not enough to reach the height of the control and that of the shorter drought treatment (S1), which also exhibited intensive growth during this period (increases of 46.8% and 10.9%, respectively). Therefore, a significant difference was still registered between them. At the same time, S2 approached the height of S1 and between them there was no significant difference any more from this respect. The findings are very similar to this in the case of the plant diameter and of the leaf width.

The number of branches and the length of the leaves of each plant also grew significantly during the re-hydration period and in these respects no significant difference was present any more among the treatments after the three weeks.
Fresh mass of shoots of S1 and S2 plants grew significantly (by 61.7 and 133.7%, respectively) during regeneration and in this context no significant difference was present among the treatments after the three weeks (Table 2.). Fresh root mass also grew dynamically in each group, however, the change during the re-hydration period was detected as significant only in the case of S2 (49.2% growth). As a consequence of these changes, the ratios of root and shoot mass after regeneration decreased in each treatment and were similar to each other after the three weeks.

Effect of drought on the active constituents of the drug

The content of volatile compounds did not show significant changes due to the stress treatment (Table 3). Water shortage seemed to decrease the accumulation rate in the first year but the result was the opposite in 2016, when S2 reached a higher level than samples of the other treatments.

We detected an elevated level of TPC both in the S2 (continuous stress) and S1 (sudden stress) treatments but only in the second year. In 2015 the concentration was highest in the control plants (Table 3.). Antioxidant capacity (AC) showed a tight connection with the phenolic values, and thus, findings are similar to that. In the first year the capacity was highest in the control while in the second year both stressed variants exhibited practically equal values, 18% higher than that of the control. RA content showed a significantly higher concentration in the continuously stressed S2 plants in 2015, but there were no differences among the pots in 2016 (Table 3.).

After the three-week re-hydration period each parameter increased significantly compared to the values measured after the stress period. The increases were 33-140% for EO, 22-64% for TPC, 49-67% for RA and 10-36% for AC. This time there were no significant differences registered any more among the stress treatments.

Discussion

Our results are comparable with former reports on drought stress behavior of lemon balm. A field experiment under

Table 1. Changes in physiological parameters of the plants in the experimental years at different sampling times (Mean±St.dev.).

Feature	2015			2016					
	End of stress period			End of stress period			After re-hydration		
	C	S1	S2	C	S1	S2	C	S1	S2
Water potential (MPa)	7.50 b ±0.91	9.13 a ±0.25	10.13 a ±0.63	35.48 Ab 2.37	38.59 Aa 0.94	39.80 Aa 1.65	34.0 Ab ±2.14	38.8 Aa ±1.60	38.1 Aa ±1.8
Relative water content (% f.w.)	97.3 a ±2.89	84.0 b ±1.73	88.3 b ±3.06	3.33 Ab ±0.82	3.90 Ab ±0.74	5.50 Aa ±1.17	2.55 Aa ±0.35	2.87 Ba ±0.44	3.50 Ba ±1.17
Dry matter content (%)	23.4 b ±0.97	26.8 a ±1.06	27.9 a ±0.55	86.8 Aa ±1.03	82.3 Aa ±2.00	81.00 Ba ±7.74	95.4 Aa ±4.09	93.6 Aa ±5.09	91.1 Aa ±4.32
Chlorophyll content SPAD	33.06 b ±0.79	36.5 a ±1.05	34.06 b ±2.00	25.3 Ab ±0.73	30.2 Aa ±0.32	29.9 Aab ±0.48	25.9 Ab ±1.12	28.7 Ba ±0.49	27.8 Ba ±0.89

One-way ANOVA was applied for comparison of the treatments in 2015. MANOVA was used for evaluating the effects of treatment and harvest time in 2016. Lower case letters indicate sign. diff. ($p<0.05$) among treatments at one sampling time. Capital letters indicate sign. diff. ($p<0.05$) between the same treatments in the two sampling times.

Table 2. Changes in morphological characteristics and biomass in the experimental years at different sampling times (Mean±St.dev.)

Feature	2015			2016					
	End of stress period			End of stress period			After re-hydration		
	C	S1	S2	C	S1	S2	C	S1	S2
Plant height (cm)	32.3 **a** ±3.46	27.2 b ±2.57	22.8 **c** ±2.78	26.7 Ba ± 3,71	30.1 Aa 2,92	20.5 Bb 4,11	39.2 Aa ±4,32	33.4 Aab ±6,88	28.2 Ab ±2,59
Plant width (cm)	53.9 a ±3.07	45.5 b ±4.33	36.0 c ±3.85	50.0 Aa ±6.32	46.1 Aa ±4.33	34.3 Bb ±2.49	52.8 Aa ±1.30	48.4 Aa ±2.70	40.4 Ab ±3.21
Number of branches (pcs)	20.9 a ±3.21	14.3 b ±1.77	13.2 b ±2.49	14.6 Aa ±1.90	13.6 Aa ±1.58	11.2 Bb ±1.40	15.6 Aa ±1.67	14.0 Aa ±2.00	13.2 Aa ±1.09
Leaf length (cm)	5.9 a ±0.72	6.0 a ±0.67	5.1 b ±0.73	5.1 Aa ±0.43	4.3 Ab ±0.58	3.7 Bc ±0.41	4.6 Aa ±0.70	4.0 Aa ±0.56	4.4 Aa ±0.62
Leaf width (cm)	4.7 a ±0.46	4.8 a ±0.40	4.0 b ±0.42	3.4 Aa ±0.40	2.7 Bb ±0.25	2.5 Bb ±0.27	3.6 Aa ±0.33	3.3 Aab ±0.25	3.2 Ab ±0.25
Fresh mass of shoot (g/plant)	123.6 a ±25.17	76.3 b ±17.3	52.5 c ±7.52	130.0 Aa ±31.0	128.0 Ba ±2.00	75.3 Bb ±9.45	193.7 Aa ±52.53	207.0 Aa ±37.24	176.0 Aa ±14.73
Fresh mass of root (g/plant)	88.3 a ±15.0	52.7 b ±3.21	43.0 b ±10.53	118.7 Aa ±51.62	96.0 Aa ±4.00	88.0 Ba ±16.0	165.0 Aa ±58.59	138.0 Aa ±40.00	131.3 Aa ±18.82
Root/shoot ratio	0.71	0.70	0.81	0.92	0.75	1.17	0.85	0.67	0.74

One-way ANOVA was applied for comparison of the treatments in 2015. MANOVA was used for evaluating the effects of treatment and harvest time in 2016. Lower case letters indicate sign. diff. ($p<0.05$) among treatments at one sampling time. Capital letters indicate sign. diff. ($p<0.05$) between the same treatments in the two sampling times.

Table 3. Changes in active constituents of the drug in the experimental years at different sampling times (Mean±St.dev.).

Feature	2015			2016					
	End of stress period			End of stress period			After re-hydration		
	C	S1	S2	C	S1	S2	C	S1	S2
Essential oil content (ml/100g d.w.)	0.251 a ±0.035	0.148 b 0±.001	0.143 b ±0.001	0.161 Bb ±0.003	0.162 Bb ±0.001	0.213 Ba ±0.001	0.372 Aab ±0.001	0.389 Aa ±0.030	0.284 Ab ±0.032
Total polyphenols (mg GAE·g^{-1} d.w.)	327.0 a ±13.14	295.1 b ±25.54	308.0 ab ±29.42	153.4 Bb ±13.76	208.7 Ba ±33.50	191.2 Ba ±13.33	252.5 Aa ±20.74	255.6 Aa ±41.23	233.8 Aa ±26.92
Rosmarinic acid content (% d.w.)	3.509 b ±0.192	2.911 c ±0.416	4.425 a ±0.646	2.815 Aa ±1.363	3.351 Ba ±0,426	2.777 Ba ±0.428	4.589 Aa ±0.874	4.999 Aa ±0.041	4.647 Aa ±0.749
Antioxidant capacity (mg AAE·g^{-1} d.w.)	257.9 a ±25.59	175.7 b ±45.36	227.9 a ±41.45	226.0 Bb ±16.23	266.8 Ba ±27.11	266.7 Ba ±31.24	308.5 Aa ±48.28	302.4 Aa ±17.61	293.0 Aa ±18.53

One-way ANOVA was applied for comparison of the treatments in 2015. MANOVA was used for evaluating the effects of treatment and harvest time in 2016. Lower case letters indicate sign. diff. ($p<0.05$) among treatments at one sampling time. Capital letters indicate sign. diff. ($p<0.05$) between the same treatments in the two sampling times

Table 4. Weather parameters in the experimental period of the S1 treatment (three weeks drought).

Parameter	2015 (9-29 July)	2016 (5-25 July)
Sum of temperatures (°C)	531.3	482.6
Daily mean temperature (°C)	25.3	24.1
Range of daily mean temperatures (°C)	17-31	16-29
Nr. of days with at least 30°C daily mean	6	0
Daily mean air humidity (RH %)	61.1	61.5
Range of daily mean air humidity (RH %)	54-73	48-88
Nr. of days when air humidity over 60%	7	11

Table 5. Characteristics of the soil mixture Florasca Bio "B".

pH	Salt %	Humus	NO_3-Nmg/kg	P_2O_5 mg/kg	K_2Omg/kg	Ca %
6.79	0.68	12.3	45.3	357	1270	1.98
Mg mg/kg	Fe mg/kg	Mn mg/kg	Zn mg/kg	Cu mg/kg	B mg/kg	$CaCO_3$ %
170	159	7.37	8.03	4.88	6.29	<1

Table 6. Soil water contents (as % of SWC) of treatments in both experimental years after planting (in 2016 with a three week re-generation period)

Period	S1 (Stress short)		S2 (Stress continuous)		Control	
	2015	2016	2015	2016	2015	2016
1-3 weeks (stress only for S2)	70	70	40	40	70	70
4-6 weeks (stress for S1 and S2	40	40	40	40	70	70
7-9 weeks (re-hydration of each)	-	70	-	70	-	70

Mediterranean conditions proved that the species may maintain nearly constant values of relative water content and xylematic water potential, thus avoiding severe water loss for two weeks (Munné-Bosch and Alegre, 1999).

As the result of drought the photosynthetic activity of plants might reduce, however our data did not show this phenomenon. It is in coincidence with the results of Munné-Bosch and Alegre (2003) who found that chlorophyll content of lemon balm did not decrease between 86% and 58% RWC and concluded a good protection mechanism against damage of photosynthetic apparatus. Moreover, this phenomenon may contribute to the described recovery of the plants after the stress period in the second year. Beside this, we have to consider that SPAD values are based on the green colour intensity of the leaves, which might be influenced also by the size of cells and the structure of the tissues. If cells are smaller, density of chloroplasts is larger, which may result in a darker leaf colour and higher SPAD value as it was found here in some cases. Former findings in connection with drought stress and SPAD chlorophyll values are contradictious. Rahimi et al. (2010) detected in *Plantago* species similar result to our ones while an intensive decrease of SPAD value due to drought was reported in soybean and cotton (Inamullah and Akihiro, 2005). In wheat, SPAD decreased only in case of severe drought (25% field capacity) while it did not change significantly between 60-100 % field capacity.

It can be concluded, that the measured physiological characteristics, especially water potential, relative water content and dry matter content ascertained that the water household situation of the plants in the applied three treatments were different.

The significant loss of biomass in lemon balm due to low water content (S2) of the soil is similar to former references (Ozturk et al., 2004, Farahany et al., 2009, Manukian, 2011) although exact circumstances of these experiments are hardly comparable with the present one. In contrary to the treatment when the drought lasted for six weeks, in the case of a shorter stress period (S1) the reduction of the measured parameters were not uniform in the two years. It may show that after a stable water supply the decreasing soil water content does not necessarily affect the plants as a sudden shock but physiological changes may carry on for a longer time. The length of this transition period may depend on several circumstances. Comparing the weather conditions in the two years, we could establish that under experimental circumstances even smaller differences might have influenced the stress tolerance of lemon balm. In 2015 during the 3 weeks of the S1 drought treatment daily temperature was by 1 ^oC higher, there were 6 days with average temperatures reaching 30 ^oC, and air humidity rarely exceeded 60% (Table 4). In such weather conditions the sudden withdrawal of water could induce faster physiological changes than it was detected in 2016.

The changing shoot/root ratio may reflect an intensive growth of the roots for reaching water in the drier soils, and may also be the result of the reduced shoot growth under unfavourable conditions. Formerly, in a climatic chamber experiment we also found, that water shortage (40% of SWC) resulted in a 2 fold root/shoot ratio of lemon balm compared to the control (Radácsi et al., 2016b).

Data on the recovery potential of lemon balm after drought stress have been lacking till now. Based on our results, it can be established that even after a reduction of 30% in plant height and 58% of shoot mass the process might be reversible and an improved water supply (water content 70% of SWC) accelerates growth and production reaching the values of the control plants grown under continuous optimal water supply.

Data on the effect of water supply on the accumulation of volatiles in Lamiaceae species are often contradictious. In lemon balm, Farahany et al. (2009) detected the highest EO percentages at the lowest water supply (20% field capacity). Manukyan (2011) also measured a slight increase of volatiles but only in the most severe drought situation. Ozturk et al. (2004) described a small (0.01-0.03%) increase only in the situations when the biomass decreased significantly. In the related species, in savory (*Satureja hortensis*), we found formerly that highest essential oil yield could be obtained from the plants growing under moderate drought stress (50% of SWC) condition while the control plants and plants exposed to severe water stress (30% of SWC) showed lower values (Radácsi et al., 2016a).

Unfortunately, the inappropriate definition of water dosages and/or differences of growth circumstances of the plants in these studies contribute to contradictory results. It should also be considered, that the timing of the period when water is reduced should have a basic role in the manifestation of the effect. The peltate glandular trichomes are developing and filled with volatile compounds in the early period of leaf growth and their number highly depends on the circumstances (Gershezon et al., 2000). Due to a water shortage already at the early phases of leaf development, a reduced number of glands would result in a lower volatile accumulation. On the other side, if drought stress reaches the plant in a later phase, its effect on EO yield of the already developed leaves would be limited or even a higher concentration might be detected. Matraka et al. (2010) explain that the higher EO yield obtained from drought stressed plants may be the consequence of increasing density of peltate hairs on the decreased leaf surface.

The information on the effect of water supply on the accumulation of phenolics in the case of lemon balm is very limited. During recent investigations in climatic chambers, lower SWC resulted in reduced biomass. However, the TPC and RA content were not affected by the treatments (Németh-Zámbori et al., 2016). This is practically in harmony with the present findings where no exact direction of change in phenolic accumulation could be established due to different water supply. The results of Manukyan (2011), who detected a significant increase in each of the polyphenols, the RA and the antioxidant capacity of lemon balm leaves due to decreasing substrate moisture (50-250 hPa) could not be ascertained under the circumstances of our pot experiment. In *Ligustrum vulgare* Tattini et al. (2004) measured significant decrease of polyphenols in drought stressed (40% of SWC, like in our experiment) plants, however, only in the presence of at least 35% solar radiation. In shade, the polyphenol level did not change.

Polyphenols, especially flavonoids and phenylpropanoids are usually considered as defence molecules in biotic and abiotic stress conditions (Treutter, 2010). Nevertheless, the measured concentrations do not necessarily and always reflect an enhanced synthesis of these molecules but may be the consequence of restricted dry matter accumulation and concentration effect, as well (Selmar and Kleinwächter, 2013). Altogether, a well-defined change of polyphenol accumulation or concentration in lemon balm leaves in consequence of reduced water supply could not been established in the present two-years experiment. Thus, it seems to be most likely that phenolic compounds might play a secondary role in drought tolerance of lemon balm. Based on this, the elevated values of active ingredients after the three-week re-hydration period that could be detected in each treatment might be in connection with other factors than water supply, e.g. age of the plants, temperature, etc. This aspect would be worth of further research.

Materials and Methods

Plant material and growth conditions

The experiment was conducted in Budapest in 2015 and 2016, at the Experimental Station of Szent István University. Plants were grown in pots under a transparent plastic ceiling in order to exclude natural precipitation and to maintain a controlled level of soil water content. The medium was a commercially available soil mixture (Florasca Bio "B"), consisting of 10% sand, 65% peat and 25% cattle manure compost. The main characteristics of the medium are summarized in Table 5.

Seedlings of lemon balm (*Melissa officinalis* L.) variety 'Quedlinburger niederliegende' were raised in a greenhouse and planted as three-month old plantlets into the pots (1 plant/pot). 10 and 20 pots/treatments were used in 2015 and 2016, respectively. Planting times and duration of the experiments are shown in Table 6. Air temperatures and air humidity during the experiment were registered at the level of the plants by an RHT10 Humidity and Temperature USB Datalogger (Extech Instruments, USA).

Treatments

Two treatments were implemented to create drought stress conditions. In treatment S1 the water shortage (40% saturation of SWC) was maintained for six weeks (Table 6). In the S2 treatment, after three weeks of a better water supply (70% SWC), the level was reduced and a 40% saturation level of SWC maintained for three weeks. The control plants grew in pots irrigated up to 70% of SWC throughout the experimental period. In addition, in 2016, from the 7th week of the experiment, a 3week long re-hydration period was also instituted by keeping the water supply at 70% SWC for each plant, in order to compare the regeneration behaviour of the plants (Table 6.). SWC was determined prior to the study using the gravimetric method (Reynolds 1970). Both SWC checking and irrigation were carried out three times per week, which had proved to be effective based on our previous experiences. The treatment was initiated after 3 weeks of acclimatization following planting. The experimental layout was a randomized block design with two and four blocks in 2015 and 2016 respectively. In each block there were five pots from each treatment.

Measurements on morphological characteristics and yield

At the end of the stress treatments (2015 and 2016) and also at the end of the regeneration period (2016), plant height (cm) was measured before cutting as the length of the longest shoot from the root neck to the tip of the shoot. Plant diameter (cm) was measured as a natural horizontal expansion of the shoots. Additionally, the number of main shoots (pc) on each individual were determined. The length and width of leaf (cm) was measured on randomly chosen fully expanded leaves. The measurements were carried out in 10 replicates.

For measuring the biomass, the plants were taken out of the pots, the roots were cleaned from soil particles and the plants were separated into shoot and root. Both aboveground and underground parts were weighed, and afterwards the shoot/root ratio was calculated.

Determination of physiological characteristics

The relative water content (RWC) was analysed from fully developed leaf samples of the third nodes under the top of the shoots (in 6 replicates per treatment). After determining fresh weight (FW), they were immersed in distilled water for 6 hours to estimate turgid weight (TW), and then the disks were dried at 60 °C for 24 h to measure dry weight (DW). The RWC was determined according to the following formula: RWC (%) = ((FW–DW)/(TW–DW))×100 (Weatherly 1950, Barrs 1968).

Chlorophyll content of the leaves, which was indicated by the quantification of green colour intensity, was measured using a SPAD 502Plus Chlorophyll Meter (Konica Minolta Inc., Japan). The readings were taken at the third internodes

from the shoot tip before harvesting. Three readings were made on each leaf and the arithmetic mean was calculated. This measurement was repeated on 6 plants for each treatment.

Determination of water potential ψ (ψ) was carried out on fully expanded leaves in 5 replicates/treatment, at the day of the harvest, between 9 and 10 o'clock in the morning. A mobile pressure chamber, type SKYE SKPM 1405, was applied, using nitrogen gas.

Phytochemical measurements

After drying the shoot samples at room temperature (20-25°C), the material of each harvested individual was mixed, creating a representative bulk sample for each treatment. Thick stem parts making up the lower two-thirds of the shoots were eliminated from the samples. The phytochemical analyses were carried out on this homogeneous bulk sample in three replicates.

Essential oil content: 50g of each sample was hydrodistilled for three hours in a Clevenger-type apparatus recommended by the Pharmacopoeia Hungarica VII. The essential oil content was calculated as volume (mL) of *essential* oil per 100 g of dried weight (determined in three hours, at 105°C).

Total phenolic content (TPC): For the determination of the TPC 1g powdered dried plant material was extracted by boiling 100 mL distilled water and then was allowed to stand for 24 h. Then the extracts were filtered and stored frozen until the measurements took place. The total phenolic content was determined by the modified method of Singleton and Rossi (1965) and was expressed as mg of gallic acid equivalents per g of dry weight of extract (GAE $mg \cdot g^{-1}$ d.w.).

Rosmarinic acid content (RA): 0.5g powdered dry plant material was suspended in 45 mL methanol. The suspension was boiled for 30 minutes in a water bath, cooled afterwards, and then filtered (with a 45µm filter) into a 100 mL flask. The filtrate was completed with methanol up to 50.0 mL volume. RA content was determined by the HPLC method based on the Ph. Eur. VIII section regarding *Melissae folium*.

Antioxidant capacity: The FRAP assay was performed according to the method of Benzie and Strain (1996), and the FRAP values of samples were calculated from a standard curve equation and expressed as mg ascorbic acid equivalent (AAE) $\cdot g^{-1}$ of dry extract.

Statistical analysis

The results were analysed with an IBM SPSS 22.0 statistical program. One-way ANOVA was applied for comparison of the treatments in 2015 and MANOVA was used for evaluating the effects of treatment and harvest time in 2016. Homogeneity of variances was tested by Levene's method. Depending the homogeneity of variances measurement, a *Tukey HSD* or *Games-Howel* test was used for the pairwise comparisons of the variances. Confidence level was 5%.

Conclusion

From the results of the present study on *Melissa officinalis* L. it can be established that a six week long period of water shortage (water content of 40% SWC) could threaten the cultivation of lemon balm, especially concerning the yield, while its effects on the quality of the drug are less dramatic.

In parallel with this, it was detected that even small changes in weather conditions (air humidity, temperature) could either aggravate or mitigate the influence of the soil drought and the onset and size of both physiological and morphological changes in the plant. Therefore, three week long drought period caused variable effects over years of the experiment. The study on potential recovery after the drought period showed that even after a reduction of 30% in plant height and 58% of shoot mass, the process might be reversible. Further, an improved water supply (water content 70% SWC) accelerates growth and production which equals the values of the control plants held under continuous optimal water supply.

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