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VARIABILITY OF PHENOLIC COMPOUNDS OF FOUR AROMATIC *Lamiaceae* SPECIES IN CONSEQUENCE OF DIFFERENT WATER SUPPLY

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ABSTRACT

The reactions of lemon balm, marjoram, peppermint, thyme were investigated and compared in a pot experiment, adjusting 70% and 40% of soil water capacity (SWC). Biomass, total phenolic content (TPC), rosmarinic acid content (RA) and antioxidant capacity (FRAP, DPPH) of both the shoots and roots were measured. As an universal phenomenon the water stress (40% SWC) decreased the total biomass production of all species drastically. The highest increase was observed in the shoot mass of peppermint and lemon balm (decreased from 52.6 g·plant^{-1} to 11.3 g·plant^{-1} and from 236.8 g·plant^{-1} to 58 g·plant^{-1}, respectively). The reaction of marjoram was much more moderate. The accumulation level of TPC was accelerated in the aboveground parts of the studied species, universally. The reactions in the roots were less characteristic. The largest increase of TPC was measured in the shoots of lemon balm (from 359.015 mg GAE·g^{-1} d.w. up to 412.44 GAE·g^{-1} d.w.). The reaction of marjoram was the less characteristic in this respect, as well. The parallel changes of biomass and TPC level might allow the total phenolic content to function as an adequate marker in predicting the lack of appropriate water supply. RA content showed species characteristics. Thyme, marjoram and peppermint reacted by a significant elevation (by 23–127%) of the RA content to the lack of water. The highest proportions were accumulated in shoots of the stressed thyme plants (3.45% d.w.).

Key words: antioxidant capacity, drought stress, *Majorana hortensis, Melissa officinalis, Mentha × piperita, Thymus vulgaris*, rosmarinic acid

Abbreviations: AAE – ascorbic acid equivalent, DPPH – 2,2-diphenyl-1-picryl-hydrazyl-hydrate, FRAP – ferric reducing ability of plasma, GAE – gallic acid equivalent, RA – rosmarinic acid, SWC – soil water capacity, TPC – total phenolic content

INTRODUCTION

Water shortage is a common phenomenon today in several regions [Staniak and Kocon 2015]. Similarly to any plant species, water availability has a great influence on medicinal and aromatic plants production. However, much less information is available about the effect of water supply on the accumulation of their active compounds, among others that of the polyphenols.

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Phenolic compounds significantly contribute to biological efficacy (gastrointestinal protection, anti-inflammatory, analgesic, antimicrobial and antioxidant activity) of *Lamiaceae* species [Mimica-Dukic and Bozin 2008]. At the same time numerous references showed that polyphenols, especially flavonoids and phenylpropanoids, may act as defence molecules in stress conditions of the plants [Treutter 2010].

In the recent experiment we studied the reaction of four related *Lamiaceae* species (lemon balm, marjoram, peppermint and thyme) to water supply with special emphasis on their phenolic compounds which have scarcely been investigated in connection with environmental effects until now.

Lemon balm (*Melissa officinalis* L.) is used against mild symptoms of mental stress and sleep disorders and the extracts are processed also in antiviral topical preparations [Barnes et al. 2007]. The main biologically active components are hydroxicinnamic acid derivatives and flavonoids, beside a low amount of essential oil [Carnat et al. 1998, Dastmalchi et al. 2008]. Lemon balm is cultivated all around the temperate and Mediterranean regions, however sophisticated data on the effects of environmental factors on active compounds are hardly available. About the changes in phenolics as a response on water supply only a single reference exists up to now which declared a significant increase of total polyphenols and rosmarinic acid of lemon balm plants due to decreasing substrate moisture [Manukyan 2011].

Marjoram (*Majorana hortensis* Mönch.) originates from the Eastern Mediterranean but is already widely distributed up to Finland, America and in Asiatic regions. The culinary value of the herb has been known since the antiquity and it is also appreciated as digestive aid [Hoppe 2013]. The drug contains numerous polyphenols, phenolglycosides, hydroxycinnamic acid derivatives and flavonoids [Fecka and Turek 2008, Sellami et al. 2009]. RA is an important constituent of the phenolic fraction, beside methyl-rosmarenate and apigenin [Mekinic et al. 2013, Roby et al. 2013]. Although its cultivation is possible under less intensive conditions, irrigation may enhance biomass and drug yield in Central Europe [Pank 1990, Hoppe 2013]. Unfortunately, according to our knowledge, no data exists about the influence of water supply on the accumulation of non-volatile phenolics in this species.

Among *Mentha* species**,** peppermint (*Mentha* × *piperita* L.) is one of the most popular one. The cultivation is widespread from the Far-East to America and throughout Europe. The biological activites (cholagogue, carminative, desinfectant) and aromatic, culinary properties are recognised in a wide row of different products. Peppermint essential oil is considered as main active substance, however, polyphenols are also present in considerable amounts and contribute to the properties of the plant beneficial to health [Areiras et al. 2001, Derakshani et al. 2012]. The leaves accumulate caffeic acid derivatives, mainly rosmarinic acid and flavonoids, mainly luteolin-7-O--glycoside [Guédon and Pasquier 1994, Mimica-Dukic and Bozin 2008, Fialová et al. 2012]. Peppermint requires high amount of soil moisture [Hoppe 2013]. However, the effect of irrigation has only been studied in connection with yield and essential oil [Penka 1978, Zámbori-Németh and Tétényi 1986] and there are no data on the reaction of the plant concerning the accumulation of phenolic compounds.

Garden thyme (*Thymus vulgaris* L.) is a commonly used culinary herb of Mediterranean origin possessing antispasmodic, antitussive, expectorant, bactericide and adstringent properties. Beside the main constituents of the essential oil, thymol and carvacrol, the plant accumulates flavonoids and phenolic acid derivatives like rosmarinic acid [Fecka and Turek 2008, Roby et al. 2013], as well. Accumulation of volatile phenolic compounds – primarily thymol – seems to be accelerated by warm and dry climatic conditions [Aziz et al. 2008, Pluhár et al. 2008] which characterize the indigenous habitats of thyme species. Nevertheless, changes in the level of non-volatile phenolics are less studied and findings are contradictory [Khosh-Khoi et al. 2012, Alavi-Samani et al. 2013].

Based on the above, we investigated and compared the reaction of the four species from *Lamiaceae* family to different conditions of water supply concerning biomass, accumulation of phenolic compounds and the antioxidant capacity of the drug. We studied the chemical characteristics both of the shoots and the roots in order to get a deeper insight into the phytochemical stress reactions of these species.

MATERIAL AND METHODS

Plant material and growth conditions

The experiment was conducted in Budapest, at the Experimental Station of the Corvinus University. Plants were grown in pots under transparent plastic roof to exclude natural precipitation and to maintain a controlled level of soil water content. The medium was a commercially available soil mixture (type Florasca Bio "B"), consisting of 10% sand, 65% peat and 25% cattle manure compost. Characteristics of the medium can be seen in Table 1. Each pot was installed to an equal 8 kg weight.

Three month old seedlings of lemon balm (*Melissa officinalis* L.) were purchased from a commercial nursery (Zöldpont Kft, Hungary). Marjoram (*Majorana hortensis* Mönch.) variety 'Magyar' (variety of our department) was raised from seeds at the research station in greenhouse and planted as 2 month old seedlings into the experimental pots. 10–15 cm high rooted shoots of peppermint (*Mentha* x *piperita* L.) variety 'Mexian' were taken from the stock plantation at our field and planted into the experimental pots. Thyme (*Thymus vulgaris* L.) variety 'Varico 3' (seed supplier Mediseed, Switzerland) was raised from seeds in greenhouse, cultivated afterwards in open field in containers and used as 7 month old young plants for the experiment.

10–10 pots/species were used, 1 plant/pot was planted for lemon balm and thyme while 2 plants/pot were installed in the case of marjoram and lemon balm. The planting was made on 21st of May 2014 and the harvest was carried out on 3rd of September 2014. At this time lemon balm and thyme were in vegetative phase while peppermint and marjoram were in full bloom.

Air temperatures and air humidity during the experiment at the level of the plants were registered by a RHT10 Humidity and Temperature USB Datalogger (Extech Instruments, USA). Daily mean temperatures fluctuated between 15.7 and 28.8°C, air humidity was 45–75%.

Treatments

Two different levels of water supply were used to ensure 40% (stressed = S) and 70% (control = C) saturation of soil water capacity (SWC). 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 was proved to be effective according to our previous experiences. The treatment was initiated after 3 weeks of acclimatization after planting.

Measurements

Root and shoot masses. At the end of the experiment the plants were taken out of the pots, the roots were cleaned from soil particles and the plants were separated into shoot and root. In the case of peppermint, the underground parts contained also the stolons of the plants. Both overground and underground parts were weighed in 5 (lemon balm, thyme) or 10 (marjoram, peppermint) replications. After that, the shoot/root ratio was calculated.

Total phenolic content (TPC). For the determination of the total phenolic content 1 g dried and powdered plant material was extracted by 100 mL boiling distilled water and was allowed to stand for 24 h. Then the extracts were filtered and stored in freezer until the measurements took place.

The total phenolic content was determined by the modified method of Singleton and Rossi [1965]. Sample solution of 0.5 mL was introduced into a test tube and then 2.5 mL Folin–Ciocalteau's reagent (10 v/v%) was added. After 1 min of incubation, 2 mL of sodium carbonate (0.7 M) was added. The

Table 1. Characteristics of the soil mixture Florasca Bio "B"

pH	Salt	Humus	Ca	$CaCO_3$	NO_3-N	P_2O_5	K_2O	Mg	Fe	Mn	Zn	Cu	B
		(%)			($mg \cdot kg^{-1}$)								
6.79	0.68	12.30	1.98	<1.00	45.30	357.00	1270.00	170.00	159.00	7.37	8.03	4.88	6.29

absorbance was measured at 760 nm in a Thermo Evolution 201 spectrophotometer after a 5 min incubation period in hot water (50°C). Gallic acid (0.3 M) was used as chemical standard for calibration. The total phenolic content of the sample was expressed as mg of gallic acid equivalents per g of dry weight of extract (GAE $mg \cdot g^{-1}$ d.w.). Blank was prepared to contain distilled water instead of extract. The measurements were carried out in 3 replications.

Rosmarinic acid content (RA). 500 g powdered dry plant material was suspended in 45 mL methanol. The suspension was boiled for 30 min in water bath, cooled after that and filtered (by 45µm filter) into a 100 mL flask. The filtrate was completed by methanol to 50.0 mL volume.

Rosmarinic acid content was determined by HPLC method in three replications. The Waters HPLC system consisted of a 1525 binary pump with a 717plus autosampler, a Jetstream column termostate and a 2998 PDA detector, controlled by Empower2 software. A Kinetex C-18 column was used, 100 mm L 4.6 mm i.d., 2.6 µm particle size. All solvents were HPLC grade. For the elution, 1 : 19 : 80 phosphoric acid : acetonitril : water (mobile phase A) and 1 : 40 : 59 phosphoric acid : methanol : acetonitrile (mobile phase B) were used as solvents at a flow rate of 1 mL min^{-1} based on the Ph. Eur. VIII section about *Melissae folium*. The gradient program started at 100% A and after solvent B was increased linearly and reached 35% in 10 min, then 100% in 2 min. Finally, 100% A was reached at 2 min. Eight min post-time was set for the equilibration of the initial solvent composition. The column temperature was maintained at 35°C and the injection volume of 5 µL was used in all experiments.

Antioxidant activity (FRAP and DPPH methods). The FRAP assay was performed according to the Benzie and Strain [1996] procedure with slight modifications. 1 g dried and powdered plant material was extracted by 100 mL boiling distilled water and was allowed to stand for 24 h. Then the extracts were filtered and stored in freezer until the measurements. FRAP reagent was prepared freshly to contain sodium acetate buffer (pH 3.6), TPTZ (2,4,6-tripiridil--s-triazin) in HCl and $FeCl_3*6H_2O$ solution (20 mmol/L), in proportion 10 : 1 : 1 (v/v/v), respectively. 10 µL of test sample was added to 1.5 mL of acting FRAP reagent and 40 µL distilled water and absorbance was recorded at 593 nm after 5 min using the above mentioned spectrophotometer. Blank was prepared to contain distilled water instead of extract. Ascorbic acid was used as positive control. FRAP values of samples were calculated from standard curve equation and expressed as mg ascorbinic acid equivalent (AAE) $\cdot g^{-1}$ of dry extract.

The antioxidant activity was determined also by using the free radical DPPH assay [Brand-Williams et al. 1995]. Sample extract preparation: 0.5 g dried plant material was extracted by 20 mL pure ethanol (96%) and shaken at 70°C for 20 min. It was then further diluted by ethanol (96%) to 50 mL, shaken and stored in freezer until the measurements took place.

The DPPH solution was prepared by weighing 2.4 mg of the ($6 \cdot 10^{-5}$ mol L^{-1}) DPPH and dissolving it in 100 mL methanol. The absorbance of the DPPH solution without sample was measured to determine the zero-time absorbance (A_0) at 517 nm using the spectrophotometer mentioned above.

For the measurement 0.1 mL of plant extract was reacted with 3.9 mL of the DPPH solution, shaken vigorously it was allowed to stand in the dark for 20 min at 20°C. After that, the decrease in absorbance of the resulting solution (A_{20}) was determined against a blank of methanol. Ascorbic acid was used as positive control. The percentages of inhibition of the DPPH radical were calculated by using the following equation: $IC\% = (A_0 - A_{20})/A_0 \times 100\%$. The IC_{50} values ($\mu g \cdot mL^{-1}$) (concentrations of the test samples and standard antioxidant providing 50% inhibition of DPPH radicals) were calculated from DPPH absorption curve at 517 nm.

Statistical analysis. The results were analysed with the IBM SPSS Statistics 22 software. In case of each species and plant organ one-way ANOVA was used to evaluate statistical differences among treatments. Normality of the residuals was proved according to the Kolmogorov-Smirnov method. No transformation of original data was needed. Homogeneity of variances was tested by Levene's method. Treatments were separated by Games-Howell's or Tukey's post hoc tests, depending on whether homogeneity assumption was violated or not.

RESULTS AND DISCUSSION

Lower SWC resulted in a significant decrease of the biomass of lemon balm: the shoot mass of the C plants surpassed that of the S ones by more than four times and the mass of the roots was almost five times higher, as well (tab. 2). At the same time, the ratio of the roots and shoots changed only slightly and higher ratio was observed in the C individuals.

Phenoloids could be detected in the whole plant, although the concentrations were considerably higher in the shoots than in the roots. According to our expectations, the TPC values were significantly higher in the S treatment compared to the C plants. This is true for both plant organs, however, the statistical probability level is higher in the roots ($p < 0.000$) than in the shoots ($p = 0.024$).

RA accumulated mainly in the shoot parts, the values in the roots are only appr. 7% of those in the shoots. Dryer soil resulted in decreasing accumulation of RA. The effects of the different water supply treatments, however, could be statistically justified only in the root samples ($P = 0.005$), (tab. 2).

Table 2. Characteristics of the control and stressed lemon balm plants

Parameter	Shoot				Root			
	Control	Stress	F value	Sign	Control	Stress	F value	Sign
Fresh mass ($g \cdot plant^{-1}$)	236.8	58.0	100.837	0.000	124.4	26.0	55.342	0.000
Total phenolics (mg GAE$\cdot g^{-1}$ d.w.)	359.015	412.440	6.202	0.024	64.417	76.596	19.027	0.000
Rosmarinic acid (% d.w.)	3.004	2.433	3.637	0.129	0.215	0.167	29.965	0.005
Antiox. cap. FRAP (mg AAE$\cdot g^{-1}$ d.w.)	222.249	315.210	92.032	0.000	26.859	28.113	0.509	0.486
Antiox. cap. DPPH (IC_{50})	1.036	0.981	5.131	0.086	0.964	0.980	1.995	0.231
Root/shoot mass ratio	0.53	0.45						

Table 3. Characteristics of the control and stressed marjoram plants

Parameter	Shoot				Root			
	Control	Stress	F value	Sign	Control	Stress	F value	Sign
Fresh mass ($g \cdot plant^{-1}$)	41.0	21.3	97.978	0.000	2.77	2.35	0.134	0.726
Total phenolics (mg GAE$\cdot g^{-1}$ d.w.)	229.17	238.03	9.028	0.008	196.33	229.65	54.837	0.000
Rosmarinic acid (% d.w.)	1.956	2.936	45.125	0.003	0.249	0.566	65.784	0.001
Antiox. cap. FRAP (mg AAE$\cdot g^{-1}$ d.w.)	216.99	232.79	8.127	0.012	157.46	220.15	161.115	0.000
Antiox. cap. DPPH (IC_{50})	1.274	1.029	70.817	0.001	0.954	0.930	0.813	0.418
Root/shoot mass ratio	0.07	0.11						

Evaluating the antioxidant capacity of the plants it could be established that in the shoots a stronger antioxidant activity was shown in the S plants by both methods (tab. 2) than in the C ones. We detected a significantly higher antioxidant capacity by the FRAP method and a slight change by the DPPH method ($p = 0.086$). This reaction was only significant in the shoots.

In several publications, a strong correlation is described between the concentration of phenolic compounds and the antioxidant capacity of different plant samples [e.g. Rusaczonek et al. 2007, Alizadeh et al. 2011, Mekinic et al. 2013]. In lemon balm, Manukyan [2011] detected a significant increase in each of the polyphenols, the rosmarinic acid and the antioxidant capacity of lemon balm leaves due to decreasing substrate moisture (50–250 hPa). Based on the above, our results are only in partial coincidence with these reports.

Marjoram produced significantly larger mass of shoots in the pots of higher SWC (tab. 3) while the mass of the roots was only slightly larger in the case of these plants. Consequently, the ratio between the biomass of roots and shoots increased in the pots of lower water supply.

It was established that both the aboveground and underground organs of marjoram contain phenolic compounds in considerable concentrations. The level of TPC was significantly increased by S treatment both in the shoots and roots. The difference is especially remarkable in case of the roots (17% increase).

Accumulation of RA was detected in the whole plant, although its concentration in the roots was only 12–20% of that in the shoots. In both organs, the lower SWC resulted in a significant increase ($p < 0.01\%$) of RA, similarly to the TPC values. The level of polyphenols in marjoram may fluctuate significantly with growth stages [Sellami et al. 2009], however, no reference is known about the influencing effect of the environment. The presence and accumulation level of polyphenols in the roots have not been published either.

The stressed plants showed a significantly higher antioxidant activity. Applying the FRAP method these differences could be justified in both organs. In the case of shoots the result was significant with the DPPH method, as well (tab. 3). Our data may ascertain the statements of Alizadeh et al. [2011] on a strong positive correlation between phenolic content (FRAP) and antioxidant activity (DPPH) in *Origanum majorana* shoot samples cultivated in Iran. Nevertheless, no connection with environmental effects and organic distribution was studied.

In line with our expectations, the biomass production of peppermint plants was significantly reduced under the S conditions (tab. 4), in harmony with former findings in open field [Zámbori-Németh and Tétényi 1986]. More than 4.5 times higher shoot yield and 1.75 times higher root mass was achieved in the C pots than in the S ones. Due to these changes, the ratio of the overground and underground organs is considerably higher in the S plants than in the C ones (3.40 and 1.28, respectively).

According to our results, the S individuals accumulated significantly higher TPC amounts than did the C plants. However, it is only demonstrated in the shoot samples. In the underground parts, an opposite tendency could be detected: the TPC level decreased due to the drought treatment (tab. 4).

The content of RA increased in peppermint due the applied stress treatment. 1.24 times higher levels were measured in the shoot samples originating from S plants compared to the C ones. In the underground organs a 1.72-fold increase could be registered in the S treatment.

The FRAP antioxidant activity of the shoots was significantly elevated by the reduced water supply. At the same time, this characteristic showed an opposite tendency in the roots (tab. 4). This is in harmony with the changes measured in TPC content.

Interestingly, the DPPH test justified a significant difference ($p = 0.045$) only in the root samples. Nevertheless, our data did not support the findings of Fialova et al. [2012] mentioning that in peppermint DPPH scavenging activity was two times higher in rhizomes than in leaves.

Table 4. Characteristics of the control and stressed peppermint plants

Parameter	Shoot				Root			
	Control	Stress	F value	Sign	Control	Stress	F value	Sign
Fresh mass ($g \cdot plant^{-1}$)	52.6	11.3	28.552	0.000	67.4	38.5	8.973	0.008
Total phenolics (mg GAE$\cdot g^{-1}$ d.w.)	199.232	236.32	79.073	0.000	92.41	71.069	850.229	0.000
Rosmarinic acid (% d.w.)	0.772	0.963	34.973	0.004	0.324	0.559	237.470	0.000
Antiox. cap. FRAP (mg AAE$\cdot g^{-1}$ d.w.)	172.795	212.91	14.249	0.002	62.71	54.055	2089.863	0.000
Antiox. cap. DPPH (IC_{50})	1.223	1.162	2.798	0.170	1.043	1.116	8.328	0.045
Root/shoot mass ratio	1.28	3.4						

Table 5. Characteristics of the control and stressed thyme plants

Parameter	Shoot				Root			
	Control	Stress	F value	Sign	Control	Stress	F value	Sign
Fresh mass ($g \cdot plant^{-1}$)	40.4	12.0	11.764	0.009	19.6	6.4	5.198	0.052
Total phenolics (mg GAE$\cdot g^{-1}$ d.w.)	266.31	317.545	50.080	0.000	249.19	199.33	155.155	0.000
Rosmarinic acid (% d.w.)	1.785	3.450	25.084	0.007	0.211	0.473	2833.927	0.000
Antiox. cap. FRAP (mg AAE$\cdot g^{-1}$ d.w.)	179.36	226.229	229.332	0.000	123.99	177.17	934.496	0.000
Antiox. cap. DPPH (IC_{50})	1.252	0.927	188.906	0.000	0.969	1.160	93.408	0.001
Root/shoot mass ratio	0.49	0.53						

Although common thyme is considered as a drought tolerant species, biomass production of the C plants surpassed the S ones by more than 200%. A more vigorous growth was reflected in the case of both the shoots and the roots (tab. 5). The root/shoot ratio increased only slightly in the S treatment. The accumulation of phenolic compounds (TPC) reflected the effect of our treatments significantly in the whole plant. However, while the TPC increased in the shoots of the S plants compared to the C ones, it decreased significantly in the roots due to the lower water supply (tab. 5).

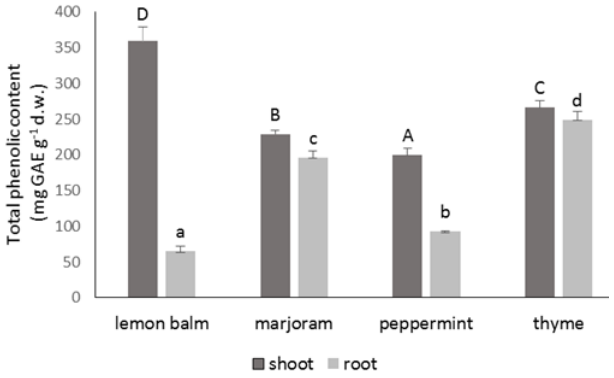
The level of RA of thyme was affected significantly by the water content of the soil. The direction of change in the accumulation of this compound proved to be similar in the whole plant. In the S pots RA content of the shoot samples increased by 93% while that of the roots was elevated by 43%.

The values demonstrating the antioxidant capacity of the plant extracts showed significant differences according to the water supply. The scavanging activity of each sample was higher in the S plants compared to the C treatment, parallel with the TPC contents. The only exemption is the result of the DPPH

assay of root samples which showed an opposite tendency (tab. 5). These findings support the data of Rusaczonek et al. [2007] reporting a close correlation between TPC and scavanging activity of thyme and other related species. Nevertheless, the authors did not examine the root of the plants.

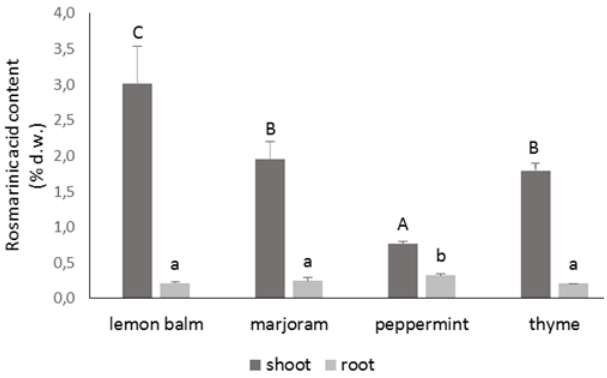
The biomass production of each of the examined species reflected the stress effect of lower water supply (S treatment), (tabs 2–4). The largest difference in the total biomass due to the treatments was detected in lemon balm (4.3-fold), followed by thyme (3.3-fold) while the ratios of peppermint and marjoram are close to each other: 2.4 and 1.9, respectively.

Although there is a limited number of references on the reaction of the studied species, the majority of references describes a decrease of biomass due to low or irregular water supply [Zámbori-Németh and



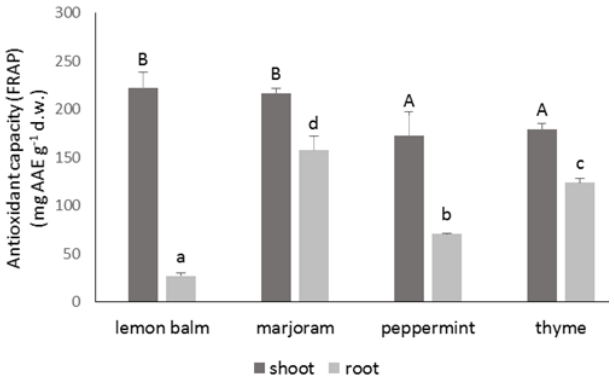
Values signed by the same lower case (in the case of roots) or capital letters (in the case of shoots) are not significantly different ($P < 0.05$) according to the post-hoc test. All values are means (±SD)

Fig. 1. Total phenolic content (mg GAE·g^{-1} d.w.) in shoots and roots of the examined species (control plants)



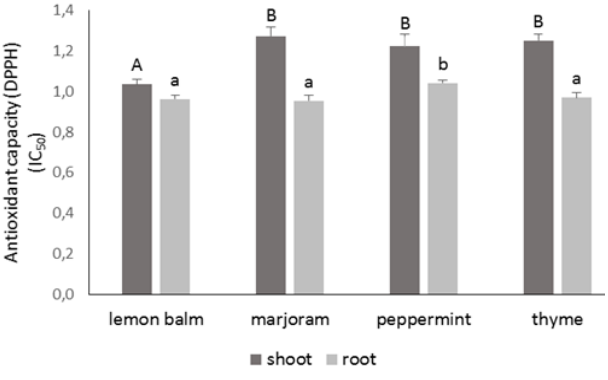
Explanations as in the Figure 1

Fig. 2. Rosmarinic acid content (% d.w.) in shoots and roots of the examined species (control plants)



Explanations as in the Figure 1

Fig. 3. Antioxidant capacity (mg AAE·g^{-1} d.w.) determined by FRAP method of the shoots and roots of the examined species (control plants)



Explanations as in the Figure 1

Fig. 4. Antioxidant capacity (IC_{50}) determined by DPPH method of the shoots and roots of the examined species (control plants)

Tétényi 1986, Zámbori-Németh et al. 2005, Manukyan 2011, Bahreininejad 2014] but opposite findings were also published [Khazaie et al. 2008]. Unfortunately, the experimental conditions of these studies are hardly comparable with the present ones. Nevertheless, to some extent, our mentioned results do not seem to be in full harmony with the fact that in the practice peppermint is considered as specifically water demanding species not advised to cultivate without irrigation [Hoppe 2013] and thyme, on the contrary, is a well-known xerophyte [Pluhár et al. 2008]. It may reflect a largely different adaptability potential of the target species. It was established that both the underground and aboveground organs contain phenolic compounds; however, the level is characteristic to the species. TPC of the four species was significantly different both in the shoots and the roots (fig. 2). Concerning the shoots, the highest levels were measured in lemon balm and the lowest ones in peppermint. However, the roots accumulated the largest concentrations in thyme and the lowest ratios in lemon balm. The accumulation in the shoots and roots showed the same magnitude both in marjoram and thyme while the accumulation level of the roots in lemon balm and peppermint was by a magnitude lower compared to the level in their shoots. The results indicate that the TPC measured in the aboveground parts presumably does not reflect the total biosynthetic potential of the plant. Formerly, both Alizadeh [2011] and Mekinic et al. [2013] measured significantly higher TPC levels in marjoram compared to thyme and lemon balm. Similarly, Roby et al. [2013] reported 1.55 times higher TPC concentrations in methanol extracts of marjoram than in that of thyme. In another study, however, almost similar TPC was determined in peppermint, thyme and lemon balm [Derakshani et al. 2012]. Underground parts of these species were not included in any of these examinations. The decreased water content of the soil increased the TPC of the shoots significantly in each species (tabs 2–5). On the other side, it was found out that the reaction of the stressed roots may be a special one characteristic to the species. While TPC of root samples was strongly elevated by the lack of water in lemon balm and marjoram, the values were decreasing significantly in peppermint and thyme. The opposite reaction of the accumulation of TPC in roots and shoots in these latter two species raises the question about translocation processes between shoots and roots in consequence of water supply although several studies indicate that stress-induced phenylpropanoids usually accumulate in the cells in which they are synthesized [Dixon and Paiva 1995]. Different defence strategies of different plant organs of *Ziziphus lotus* were concluded by Maraghni et al. [2014] the shoots accumulating mainly solutes while the roots increasing their antioxidant activity in drought conditions.

The concentration of RA in the shoots was determined between 0.772% (peppermint) as minimum and 3.450% (lemon balm) as maximum value (tabs 2–5). From among 14 different Portugese samples of peppermint 12 ones showed higher values than the samples of our experiment [Areias et al. 2001]. The high level of RA in lemon balm compared to other species was detected in other studies, as well. Janicsák et al. [1999] reported RA contents in lemon balm up to 4.40 mg/g being one of the highest among the investigated 76 *Lamiaceae* species. No significant difference was found in our trial between the RA content of marjoram and thyme (fig. 3) although former references showed almost double values in the latter species [Fecka and Turek 2008]. Different results were obtained also by Mekinic et al. [2013] who described the highest RA content in lemon balm (2500 $mg \cdot L^{-1}$), a somewhat lower value in marjoram (2000 $mg \cdot L^{-1}$) and by a magnitude lower concentration in thyme (600 $mg \cdot L^{-1}$) in ethanol/water extracts of these species.

In contrast with the TPC described above, distribution of RA inside the plant does not reflect characteristic specialities of the target species (fig. 3). Each studied species accumulated RA both in its shoots and roots. However, the accumulation level of the roots lagged far behind the levels of the shoots. The difference among the organs was the highest in the case of lemon balm (14-fold difference) and the smallest in peppermint (2.4-fold difference). According to our knowledge, accumulation of RA in the roots has not been reported till now in these species.

Evaluating the responses of the species on the lower SWC it could be established that three of them

(marjoram, peppermint, thyme) reacted by a significant elevation of the RA accumulation level in the whole plant (tabs 3–5). On the contrary, lemon balm shoots did not show significant changes in consequence of the treatment. Moreover, in its roots, the level of RA decreased in S samples (tab. 2).

Lemon balm had significantly higher scavenging capacity than the other species (figs 4–5). The antioxidant capacity of marjoram was also high, especially in the FRAP method. Similarly, to our results, in a recent study Mekinic et al. [2013] determined the highest scavenging activity in lemon balm and marjoram samples, which were both considerably higher (1.6- and 1.9-fold, respectively) than the values in thyme. According to other data on comparing IC_{50} values, thyme was only the third and lemon balm the fourth one after oregano and rosemary [Rusaczonek et al. 2007]. In this respect, Roby et al. [2013] reported only slight differences among the investigated *Lamiaceae* species (thyme, sage and marjoram).

As for antioxidant values of the roots, FRAP method proved that each studied species was statistically divergent from the other ones. The highest values (157.460 mg GAE$\cdot g^{-1}$ d.w.) were registered for marjoram and the lowest ones in lemon balm (26.859 mg GAE$\cdot g^{-1}$ d.w.). The low scavenging activity of peppermint roots was approved also by the IC_{50} values while this method did not reflect difference among the root samples of the other three species (figs 4–5). In the case of marjoram and thyme, both organs (shoots and roots) showed comparable values with each other while in peppermint and lemon balm the concentrations in the roots are much lower than those of the shoots. According to our knowledge, there are no previous reports about the scavenging activity of root extracts of these species.

The effect of lower water supply influenced the antioxidant activity of the samples in each species significantly – except the root samples of lemon balm. The direction of the change was similar to that of the results on the TPC: The S treatment resulted in elevated antioxidant activity in all shoot samples while the reaction of the roots may be characteristic to the species (tabs 2–5).

CONCLUSION

1. Phenolic content, its organic distribution and stress reaction of the related experimental species are in several respect different. In the case of lemon balm and peppermint lower soil water content resulted in a large decrease of biomass while TPC, RA and antioxidant capacity tend to changed only moderately.

2. Thyme and marjoram showed significant changes in increasing TPC, RA and antioxidant capacity values and could maintain their biomass production with a less severe loss.

3. It can be concluded, that phenolic compounds might play a secondary role in drought tolerance of lemon balm and peppermint while it presumably plays a more important role in marjoram and thyme. The especially high levels of TPC and RA in the roots might contribute to the this in the latter species.

4. Maintaining proper water supply in field cultivation of lemon balm and peppermint would be of high importance while our results ascertained the practical findings about the good drought tolerance of marjoram and thyme.

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