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Effect of Growing Factors on Productivity and Quality of Lemon Catmint, Lemon Balm and Sage under Soilless Greenhouse Production: I. Drought Stress

Artur Manukyan

Center of Greenhouses and Laboratories Dürnast, Center of Life Sciences Weihenstephan, Technical University of Munich, Dürnast 4, 85350 Freising-Weihenstephan, Germany

Corresponding author: * manukyan@wzw.tum.de

ABSTRACT

The formation of bioactive constituents of plants depends on the actual environmental and on growing factors. Controlled environment technology ensures the application of specific stresses, such as drought stress, that can optimize the production of secondary plant metabolites by inducing natural biochemical changes in plants. The impact of drought stress on herbal yield, content and composition of essential oils and polyphenols, as well as on antioxidative capacity of polyphenol-rich extracts of lemon catmint (*Nepeta cataria* L. *f. citriodora*), lemon balm (*Melissa officinalis* L.) and sage (*Salvia officinalis* L.) was studied under soilless greenhouse cultivation. It was found that the tested substrate moistures (50, 125, 250 hPa) had a considerable effect on herbal yield of lemon catmint, lemon balm and sage with maximum raw medicinal material at 50 hPa. Drought stress influenced the essential oil content of lemon catmint and lemon balm, but not of sage, with 250 hPa resulting higher amount of essential oils. 50 hPa provided high yield of essential oil for all three species. The essential oil composition of studied herbs was mainly affected by tested substrate moistures. The influence of drought stress was also significant for the content of polyphenols in lemon balm and sage, but not in lemon catmint. High drought stress (250 hPa) affected positively polyphenolic content in lemon balm, while in sage polyphenols reached maximum at 125 hPa. HPLC analysis of polyphenols showed differences in sensitivity of main polyphenols in studied herbs to drought stress factor. Polyphenol-rich extracts of lemon catmint and lemon balm showed no differences in antioxidative capacity by the ABTS system, while for sage there were small differences.

Keywords: bioactive compounds, hydroponics, *Melissa officinalis*, *Nepeta cataria f. citriodora*, *Salvia officinalis*, substrate moisture

INTRODUCTION

Soilless greenhouse production of medicinal and aromatic plants is a perspective and economical alternative for high productivity, superior quality with ecological sound and ensures that the plants free from biotic and abiotic contaminations with consistent biochemical profiles. For these purposes an adapted technology, including information on effects of stress factors, such as drought stress, is required. Growing and environmental factors play a major role in management of herbal yield and quality providing medicinal plant material with the use of natural tools (Franz 1983; Hornok 1986; Bettray *et al.* 1992; Manukyan 2004a, 2004b; Zobayed *et al.* 2005; Khalid 2006; Manukyan *et al.* 2006; Belitz *et al.* 2007; Koocheki *et al.* 2008; Razmjoo *et al.* 2008; Farahani *et al.* 2009; Said-Al Ahl *et al.* 2009; Baghalian *et al.* 2011; Li *et al.* 2011). The optimal technology that will allow producing high quality plant material with minimum amounts natural resources (e.g. water) is environmentally sound and highly necessary for modern production process (Schnitzler *et al.* 2003). As for plant production in general, it is likewise true for growing of medicinal and aromatic plants. The aim of this study is to investigate the effect of drought stress on herbal yield, amount and composition of essential oils (EOs) and flavonoids, as well as on antioxidative capacity of flavonoid-rich extracts of some valuable medicinal plants under soilless greenhouse conditions. Such information provides a basis to modify and optimise the quality of medicinal and aromatic plants, using conventional plant breeding combined with manipulation of agronomic practices.

MATERIALS AND METHODS

Plant material and experimental design

The experiments were conducted at the Center of Greenhouses and Laboratories Dürnast of the Center of Life Sciences Weihenstephan, Technical University of Munich. Medicinal and aromatic plants to study were lemon catmint (*Nepeta cataria* L. *f. citriodora*), lemon balm (*Melissa officinalis* L.) and sage (*Salvia officinalis* L.) - (all three species belong to the Lamiaceae family). Cultivation was started by sowing seeds, derived from hydroponically grown plants, in common peat ("Floraton 3") in a green-

Fig. 1 Medicinal and aromatic plants under soilless greenhouse conditions.

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house. Seeds were stratified by application of 2 g/l KNO_3 solution (Merck) and 10°C for one week. Pricking was done one month later into cell trays with common peat substrate ("Floraton 2"). The experiment started another month later with transplanting of the seedlings into 3 l (lemon catmint and sage) and 5 l (lemon balm) pots. The plants were cultivated in automatically controlled greenhouse (**Fig. 1**). For all species uncomposted wood fibre "Toresa special (Ts)" was used as substrate, which had pH 5.9 and contained 0.8 g/l water-soluble salts (233 mg/l N, 9 mg/l P_2O_5 and 90 mg/l K_2O). For each species 12 ebb-and-flood tables were used with 3.3 m^2 surface area for each with 9 plants per m^2 (30 plants per treatment with 4 repetitions). The nutrient solution was made up from 70 g "Flory" basis 1 (P-43; K-220; Mg-21; B-0.1; Cu-0.0015; Fe-1; Mn-0.2; Mo-0.003; Zn-0.0025 mg/l) in addition to 150 mg/l N (composed of 21.4 g ammonium nitrate (Merck) and 48.2 g calcium nitrate (Merck) per 100 l of water) with pH of 5.3-7.2 and electrical conductivity (EC) of 1.7-3.5 mS/cm. The irrigation of plants was performed via tensio switch sensors providing substrate moisture of 50, 125, 250 hPa. Plants were harvested at week 8 after transplanting. The criterion for lemon catmint harvest was the time of flower emergence; the other two species were harvested almost at the same time having minimum 80 (for lemon balm) and 50 g/plant (for sage) fresh biomass. The fresh medicinal material was dried at 35°C for 5-7 days.

Analyses

EO from fresh herbal material of selected medicinal and aromatic plants were extracted by steam distillation and pentane (Carl Roth) extraction for 1 h (SDE). The extracts were dried over sodium sulfate (Merck) and concentrated under a stream of nitrogen. The amount of essential oil was determined gravimetrically. The composition of the essential oil was analyzed by GC and GC-MS. The oils were diluted in acetone (Carl Roth) (split 1: 40) with separation of the compounds by GC (Fisons Instruments Mega 5360, Italy) on a Supelco-Wax capillary column (60 m, i.d. = 0.32 mm, 0.25 µm film thickness) with helium as carrier gas (0.8 ml/min) and a temperature program: 50°C (3 min), 10°C/min, 120°C (2 min), 2°C/min, 155°C (0 min), 8°C/min, 240°C. Identification of EO main compounds was performed with a GC-MS system (HP 5890 Series II/HP 5971 A, HEWLETT PACKARD) on the same column and with the same temperature program by electron impact ionization at 70 eV. Mass spectra were evaluated by comparison of retention times and mass spectra (Wiley 1990) and with an own terpenoid mass spectra database.

Polyphenols were extracted from 0.4 g dry material (powder) with 80% MeOH (Carl Roth) and separated by HPLC. Extracted polyphenols were resolved in 500 µl CH_3CN (Merck) and 500 µl EtOH (Merck), injection volume was 100 µl. The analytical HPLC was carried out on a dionex system (pump P580, autosampler Gina 50) using a 250 mm x 4,6 mm RP-18 column (phenomenex Hydro-RP) with guard column. A gradient sequence using (A) water, acetonitrile and acetic acid (Merck) (97: 2: 1) and (B) acetonitrile in the following proportions: 0% B (0 min), 50% B (5 min), 95% B (10 min), 0% B (14 min) at a flow rate of 1 ml/min. Detection and quantification were performed using a Diode Array detector (Dionex UVD 340S) and chromeleon (Dionex) software. Folin-Ciocalteau test (Merck) applied to determinate the total content of polyphenolics in plant material (Singleton *et al.* 1998). The ABTS-system was used to evaluate the antioxidative capacity of polyphenol-rich extracts (Arnao *et al.* 1999; Re *et al.* 1999). The ability to reduce the peroxidase (myeloglobin/H_2O_2)-generated ABTS•+ [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical cation] has been used to rank the antioxidant activity of various agents including dietary flavonoids and chalcones (Chan *et al.* 2003). In this system, myoglobin (Sigma) and H_2O_2 (Merck) oxidise ABTS (Merck) to the green ABTS•+ radical cation. 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical cation (ABTS•+) is a stable chromophore which absorbs strongly at 734 nm. This reaction can be followed photometrically. The lower rate of ABTS•+ formation indicates reducing or Fe-chelating properties of the extract. The analyses were performed with 3-4 replications. The statistical analyses were performed according to Dospekhov (1985).

RESULTS

The herbal yield of studied species was affected by different substrate moistures. High drought stress influenced negatively the productivity of herbs. At 250 hPa, lemon catmint, lemon balm and sage produced respectively 42.4, 25.4 and 50.9% less total raw medicinal material than at 50 hPa. In case of lemon balm and sage high drought stress of 250 hPa provided a higher percent of dry weight (**Table 1**).

Tested substrate moistures influenced the EO content of lemon catmint and lemon balm (**Fig. 2**). High drought stress (250 hPa) induced a higher amount of EO in lemon catmint and lemon balm (0.151 and 0.068%, respectively), while in the case of sage there was no significant difference between the treatments. Calculations of EO yield provided statistical differences for all three species. 50 hPa treatment was best for lemon catmint, lemon balm and sage (0.363, 0.087 and 0.198 ml/plant, respectively) (**Fig. 3**) due to the high fresh herbal biomass per plant (see also **Table 1**).

Lemon catmint EO composition was affected by the different substrate moistures under soilless greenhouse condi-

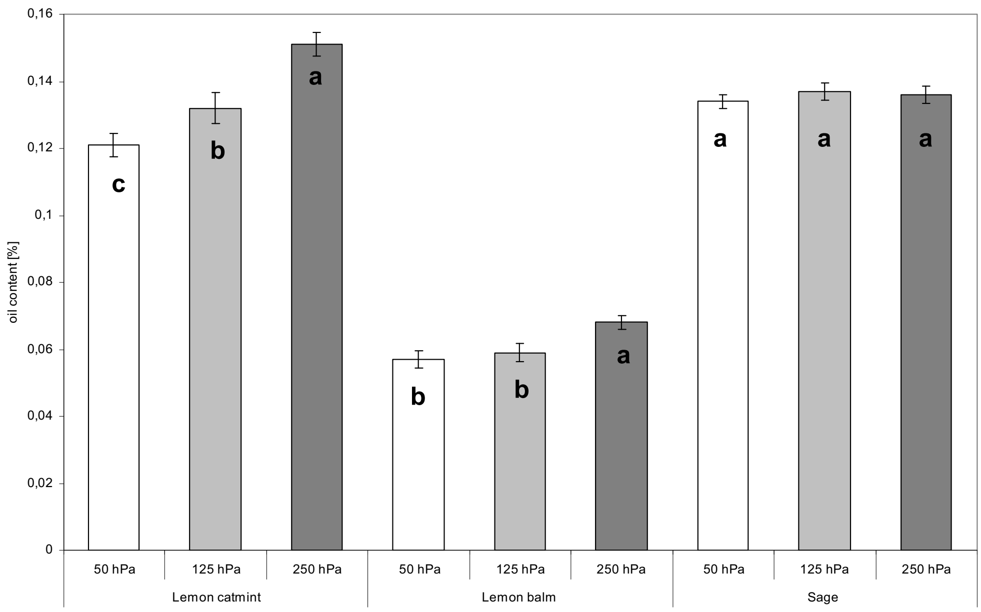

Fig. 2 Influence of substrate moisture on essential oil content of herbs. Values represent mean ± standard deviation (SD). Statistics are made within the same species.

Table 1 Influence of substrate moisture on herbal yield.

Species	Substrate moisture, hPa	Fresh medicinal material, g/plant	Raw medicinal material	
			g/plant	% (dry weight)
Lemon catmint	50	300.2 a	42.7 a	14.2 a
	125	248.0 b	32.7 b	13.2 a
	250	169.0 c	24.6 c	14.6 a
LSD_{05}*	---	36.0	5.1	1.8
Lemon balm	50	152.3 a	20.9 a	13.7 c
	125	93.7 b	15.7 b	16.8 b
	250	80.1 b	15.6 b	19.5 a
LSD_{05}*	---	18.3	2.5	2.3
Sage	50	148.0 a	21.4 a	14.5 b
	125	114.5 b	18.3 b	16.0 b
	250	57.2 c	10.5 c	18.4 a
LSD_{05}*	---	17.8	2.6	2.2

* Within the same species

Table 2 Influence of substrate moisture on lemon catmint essential oil composition.

Compound*	Substrate moisture, hPa		
	50	125	250
	Content in essential oil, %		
cis-ocimene	0.14 a**	0.13 a	0.14 a
cis-3-hexenal	0.02 a	0.02 a	0.02 a
trans-ocimene	0.01 b	0.01 b	0.02 a
cis-3-hexenyl acetate	0.01 b	0.01 b	0.02 a
6-methyl-5-hepten-2-on	1.29 a	1.08 b	1.12 b
trans-3-hexen-1-ol	0.06 b	0.07 a	0.05 c
cis-3-hexen-1-ol	2.77 a	2.76 a	2.16 b
trans-2-hexen-1-ol	0.27 b	0.35 a	0.34 a
citronellal	1.96 a	1.76 a	1.91 a
linalool	0.10 b	0.19 a	0.11 b
β-caryophyllene	1.11 ab	1.18 a	1.00 b
citronellyl acetate	0.03 a	0.03 a	0.03 a
α-humulene	0.09 a	0.09 a	0.08 a
neral	12.22 a	12.27 a	11.57 a
neryl acetate	0.02 b	0.02 b	0.03 a
geranial	15.88 a	16.03 a	15.00 a
geranyl acetate	0.07 b	0.08 b	0.11 a
citronellol	14.43 a	13.79 a	14.89 a
nerol	19.03 a	18.51 a	19.84 a
geraniol	27.67 a	28.74 a	28.52 a
β-ionone	0.01 b	0.02 a	0.01 b
phytol	0.16 c	0.46 b	0.61 a

* Only identified compounds are presented
** Statistical analyses are made within the same compound

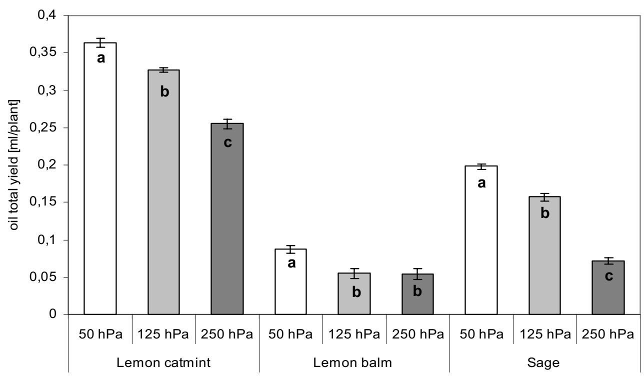

Fig. 3 Influence of substrate moisture on essential oil yield of herbs. Values represent mean ± standard deviation (SD). Statistics are made within the same species.

tions (**Table 2**). Main compounds of the EO were geraniol (27.67-28.74%), nerol (18.51-19.84%), geranial (15.00-16.03%), citronellol (13.79-14.89%) and neral (11.57-12.27%). The identified 22 compounds represented approximately 97, 98 and 98% of the total oil at 50, 125, 250 hPa substrate moisture, respectively. Drought stress had a considerable impact on the quantity of some minor compounds in lemon catmint EO, such as *trans*-ocimene, *cis*-3-hexenyl acetate, 6-methyl-5-hepten-2-on, *trans*-3-hexen-1-ol, *cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol, linalool, β-caryophyllene, neryl acetate, geranyl acetate, β-ionone and phytol. High drought stress of 250 hPa provided high content of *trans*-ocimen, *cis*-3-hexenyl acetate, neryl acetate, geranyl acetate and phytol in lemon catmint EO, while the content of 6-

Table 3 Influence of substrate moisture on lemon balm essential oil composition.

Compound*	Substrate moisture, hPa		
	50	125	250
	Content in essential oil, %		
β-myrcene	0.06 c**	0.07 b	0.08 a
cis-ocimene	0.04 c	0.12 a	0.08 b
cis-3-hexenal	0.02 a	0.01 b	0.02 a
trans-ocimene	0.20 a	0.10 c	0.14 b
6-methyl-5-hepten-2-on	1.03 c	1.28 b	1.75 a
trans-3-hexen-1-ol	0.53 b	0.82 a	0.58 b
trans-2-hexen-1-ol	0.30 b	0.40 a	0.25 b
1-octen-3-ol	0.17 a	0.16 a	0.18 a
citronellal	0.20 c	0.30 b	0.49 a
linalool	0.42 a	0.32 b	0.34 b
not identified	0.36 c	0.63 b	0.75 a
not identified	0.82 b	1.10 a	1.23 a
β-caryophyllene	0.72 b	0.85 a	0.86 a
α-humulene	0.02 b	0.04 a	0.04 a
neral	26.58 b	31.38 a	32.52 a
neryl acetate	0.17 c	0.20 b	0.24 a
geranial	35.00 b	41.23 a	42.18 a
geranyl acetate	1.47 b	1.68 ab	1.85 a
citronellol	0.29 c	0.43 b	0.60 a
nerol	3.37 a	3.01 a	3.12 a
geraniol	17.71 a	12.40 b	11.29 b
cis-9-octadecenoic acid methyl ester	0.63 a	0.13 b	0.05 c
not identified	5.91 a	1.39 b	0.01 c
not identified	1.45 a	0.36 b	0.01 c

* Only identified and not identified main substances of about or > 1% are presented
** Statistical analyses are made within the same compound

Table 4 Influence of substrate moisture on sage essential oil composition.

Compound*	Substrate moisture, hPa		
	50	125	250
	Content in essential oil, %		
α-pinene	1.58 a**	1.28 b	1.76 a
camphene	1.50 b	2.28 a	2.47 a
β-pinene	2.71 a	2.46 a	2.36 a
sabinen	0.33 a	0.35 a	0.24 b
β-myrcene	0.73 a	0.76 a	0.74 a
α-terpinene	0.13 a	0.13 a	0.13 a
limonene	0.73 a	0.66 a	0.75 a
1,8-cineole	6.30 b	4.11 c	7.97 a
cis-ocimene	0.18 a	0.20 a	0.11 b
cis-3-hexenal	0.10 a	0.03 b	0.09 a
γ-terpinene	0.32 a	0.31 a	0.31 a
p-cymol	0.06 b	0.06 b	0.07 a
terpinolene	0.24 a	0.19 b	0.19 b
trans-3-hexen-1-ol	0.59 a	0.57 a	0.41 b
trans-2-hexen-1-ol	0.20 a	0.15 b	0.09 c
α-thujone	41.44 a	42.72 a	32.10 b
β-thujone	8.62 c	11.72 b	16.17 a
camphor	15.45 ab	13.93 b	17.09 a
bornyl acetate	0.72 b	0.96 a	0.92 a
β-caryophyllene	1.94 a	1.40 b	1.28 b
α-humulene	3.59 a	2.87 b	3.34 a
borneol	1.62 b	2.60 a	1.95 b
myrtenol	0.25 b	0.37 a	0.23 b
viridiflorol	5.33 a	4.79 ab	4.33 b
manool	2.55 a	2.56 a	2.14 b

* Only identified compounds are presented
** Statistical analyses are made within the same compound

Table 5 Influence of substrate moisture on polyphenol content of herbs.

Species	Substrate moisture, hPa	Gallic acid equivalents-GAE [mM]
Lemon catmint	50	0.65 ± 0,02 a
	125	0.66 ± 0,02 a
	250	0.59 ± 0,01 a
LSD$_{05}$*	---	0.09
Lemon balm	50	1.08 ± 0,03 c
	125	1.45 ± 0,04 b
	250	1.75 ± 0,05 a
LSD$_{05}$*	---	0.23
Sage	50	1.63 ± 0,04 b
	125	2.18 ± 0,06 a
	250	1.65 ± 0,04 b
LSD$_{05}$*	---	0.32

* Within the same species

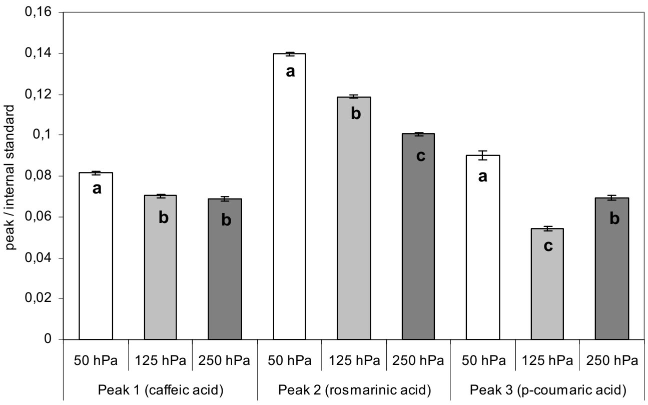

Fig. 4 Influence of substrate moisture on quantity of major polyphenols in lemon catmint. Values represent mean ± standard deviation (SD). Statistics are made within the same compound.

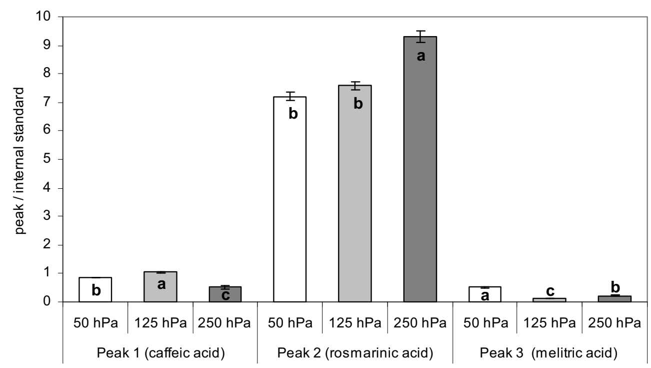

Fig. 5 Influence of substrate moisture on quantity of major polyphenols in lemon balm. Values represent mean ± standard deviation (SD). Statistics are made within the same compound.

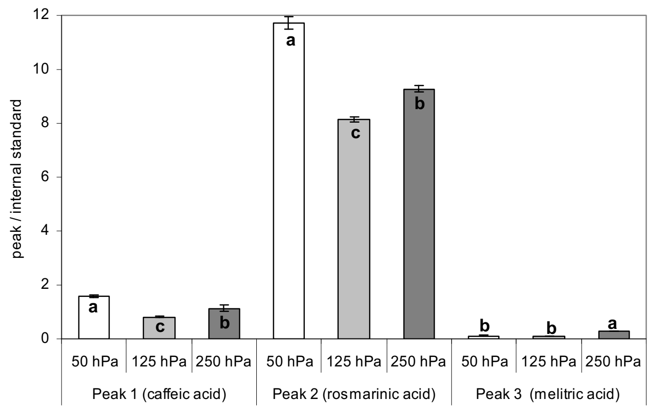

Fig. 6 Influence of substrate moisture on quantity of major polyphenols in sage. Values represent mean ± standard deviation (SD). Statistics are made within the same compound.

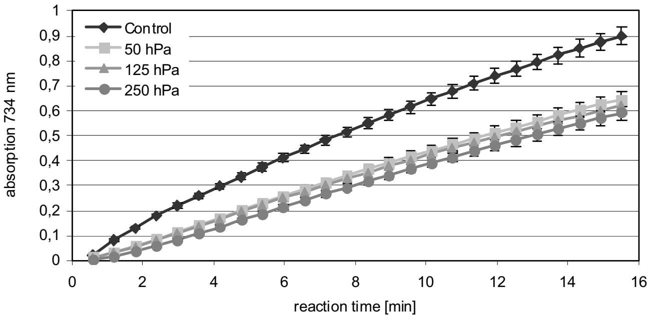

Fig. 7 Influence of substrate moisture on antioxidative capacity of polyphenol-rich extracts of lemon catmint.

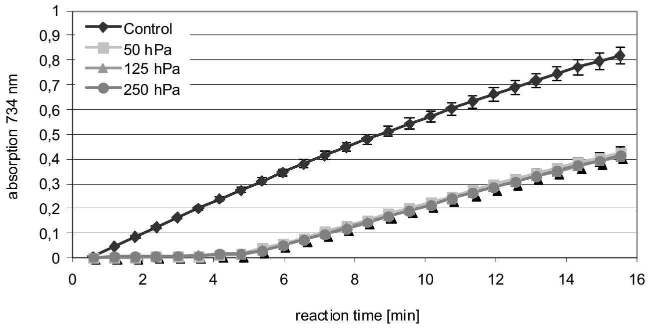

Fig. 8 Influence of substrate moisture on antioxidative capacity of polyphenol-rich extracts of lemon balm.

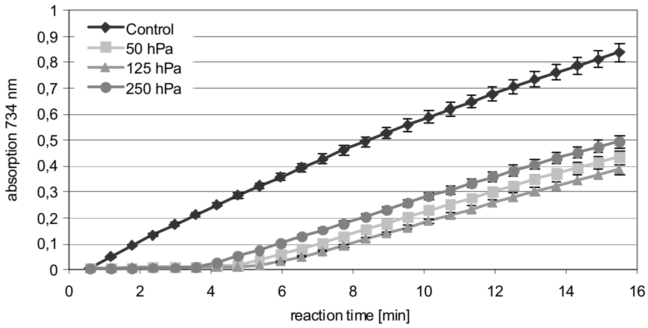

Fig. 9 Influence of substrate moisture on antioxidative capacity of polyphenol-rich extracts of sage.

methyl-5-hepten-2-on and *cis*-3-hexen-1-ol decreased. The content of *trans*-3-hexen-1-ol, linalool, β-caryophyllene and β-ionone reached maximum at 125 hPa and after that decreased again. At the same time, for the content of lemon catmint EO main compounds, drought stress factor had no significant effect.

Lemon balm EO consisted mainly of geranial (35.00-42.18%), neral (26.58-32.52%) and geraniol (11.29-17.71%) under soilless greenhouse production (**Table 3**). 20 compounds representing about 89, 95 and 97% of the total oil (at 50, 125, 250 hPa, respectively) were identified. The content of all these compounds, except for 1-octen-3-ol and nerol, depended on moisture level in used wood fibre substrate. From the main compounds of lemon balm EO, the contents of geranial and neral peaked up with increasing of drought stress, while geraniol reached a maximum when plants were grown at 50 hPa.

For sage, the 25 identified compounds of EO represented approximately 97% of the oil composition at 50, 125, 250 hPa. Among these, α-thujone (32.10-42.72%), camphor (13.93-17.09%), β-thujone (8.62-16.17%), 1,8-cineole (4.11-7.97%) and viridiflorol (4.33-5.33%) were the major constituents under soilless greenhouse conditions (**Table 4**). Drought stress had considerable influence on the content of most identified compounds in sage EO, such as α-pinene, camphene, 1,8-cineole, *cis*-ocimen, *cis*-3-hexenal, p-cymol, terpinolene, *trans*-3-hexen-1-ol, *trans*-2-hexen-1-ol, α-thujone, β-thujone, camphor, bornyl acetate, β-caryophyllene, α-humulene, borneol, myrtenol, viridiflorol and manool. From the main compounds the content of α-thujone and viridiflorol was higher at 50 and 125 hPa, while high drought stress of 250 hPa resulted maximal amount of β-thujone. The content of other two main compounds (1,8-cineole and camphor) was minimum at 125 hPa.

The influence of drought stress was also essential for the content of polyphenols in lemon balm and sage, but not in lemon catmint under soilless greenhouse conditions (**Table 5**). Lemon balm and sage contained more polyphenolic compounds than lemon catmint. High drought stress of 250 hPa was effective for biosynthesis of polyphenols in lemon balm; while for sage we have recorded a higher content of polyphenols at 125 hPa.

HPLC analysis of main polyphenols provided differences in sensitivity of main polyphenols in selected herbs to drought stress factor. In lemon catmint, all three main polyphenolic compounds (caffeic, rosmarinic and p-coumaric acids) showed high results at 50 hPa (**Fig. 4**). In lemon balm, main peak 2 (rosmarinic acid) reached maximum at high drought stress (250 hPa), while peak 1 (caffeic acid) topped at 125 hPa and peak 3 (melitric acid) at 50 hPa (**Fig. 5**). In sage, main peak 2 (rosmarinic acid) and peak 1 (caffeic acid) reached maximum results at 50 hPa, while peak 3 (melitric acid) in case of high drought stress treatment (**Fig. 6**). At the same time we have recorded that concentration of rosmarinic acid in lemon balm and sage was ca. 10 times higher than in lemon catmint.

Polyphenol-rich extracts of lemon balm and sage had higher antioxidative capacity in the ABTS-system and were tested in 10-fold lower concentrations (1: 200) (**Figs. 8-9**) than those of lemon catmint (1: 20) (**Fig. 7**). Polyphenol-rich extracts of lemon catmint grown under different substrate moisture conditions showed no essential differences in antioxidative capacity (**Fig. 7**). In case of lemon balm there were no significant differences as well and the shapes of the curves were almost synchronic (**Fig. 8**). Polyphenol-rich extracts of sage showed small differences in antioxidative capacity by the ABTS model system, particularly 125 hPa treatment with high content of polyphenols (see also **Table 5**) produced a higher antioxidative capacity (**Fig. 9**).

DISCUSSION

The intensive use of water and fertiliser in modern horticulture reflects a need for developing new and more environmental suitable production methods to reduce the usage of water and fertilizer. At the same time the improvement of inner quality of useful species is highly necessary. In this study we have focused on drought as a single stress factor. Our results showed that comparatively high drought stress (250 hPa) influenced negatively the productivity of studied herbs as awaited. At the same time some differences noted in connection to the influence of drought stress factor on herbal yield, as there were stronger differences in case of sage and lemon catmint. High drought stress affected positively EO content in lemon catmint and lemon balm, but this advantage was not enough for high yield of EO, because of low fresh herbal yield in that treatment. Drought stress mainly affected the EO composition of studied herbs under soilless greenhouse conditions. For polyphenolic content and composition of selected medicinal and aromatic plants we have seen essential influences as well, while in case antioxidative capacity of polyphenol-rich extracts we have recorded comparatively less critical impacts. This may be explained by specific inhibition potentials of some polyphenols in the extracts of selected herbs.

Several authors have published data on the effects of irrigation regime, substrate moisture and drought stress on productivity and quality of different medicinal and aromatic plants. The impact of above-mentioned factors on biosynthesis of some pharmaceutical compounds in medicinal plants was described both as positive and negative or neutral depending on certain bioactive compound and species. One research study investigated the effects of acute periods of drought stress and plant age on dry weight, and alkamide and phenolic acid content in purple coneflower roots [*Echinacea purpurea* (L.) Moench] and found that the total alkamide, including the tetraenoic acid isomers, and chlorogenic acid concentrations from fall-harvested roots were largely unaffected by drought stress, regardless of at which stage the stress occurred (Gray *et al.* 2003a). On the other hand, many other researches showed certain effect of drought stress on bioactive constitution of some herbs. For instance, in an experiment, where *Nicotiana glauca* plants were subjected to different irrigation levels, it was found that increased irrigation frequency effect negatively the alkaloids percentage in the leaves, at the same time the highest rutin percentage was found with the shorter irrigation intervals (Saleh *et al.* 1978). At the same time, drought stress effects a decrease of the indole alkaloid in *Catharanthus roseus* (Frischknecht *et al.* 1987) and influences negatively on the content of digitoxigenin in woolly foxglove *Digitalis lanata*, whereas the other cardenolides, including digoxin, were less affected (Stuhlfauth *et al.* 1987). It was also shown that in deadly nightshade (*Atropa belladonna* L.) there is correlation between the yield of tropane alkaloids (hyoscyamine and scopolamine) and irrigation regime, total nitrogen supply. The maximal yield of tropane alkaloids was achieved when plants grown under low available soil water (35%) and low nitrogen supply conditions. By contrast, the maximal content of alkaloids was achieved with 95% depletion of available soil water accompanied with high nitrogen supply (Baričevič *et al.* 2002). A number of phytochemicals in cultivated St. John's wort, including hypericin, pseudoohypericin, chlorogenic acid, rutin, hyperoside, isoquercitrin, quercitrin, and quercetin, are significantly increased being influenced by acute drought stress. Increases ranged from 5 to 36% (hyperoside and rutin, respectively). Conversely, the concentrations of hyperforin and adhyperforin were decreased by an average of 10% in drought-stressed plants as compared to well-watered plants (Gray *et al.* 2003b). The decrease in soil field capacity from 100 to 40% reduced EO yield of drought-tolerant and non-drought-tolerant clones of *Tagetes minuta* under greenhouse conditions. The drought significantly altered the content of some oil components (Mohamed *et al.* 2002). Water stress influences the EO as well as its constituents of betelvine (*Piper betle* L.), effects being depended on magnitude of drought stress (Chatterjee 1999). It resulted in significant reductions in chlorophyll, carotenoids, Fe, Mn, Zn, and EO yield of Japanese mint (*Mentha arvensis* L. cv. MS 77) (Misra *et al.* 2000). Drought and cold stress treatments caused increased in levels of (-)-epicatechin and hyperoside in *Crataegus laevigata* and *Crataegus monogyna* (hawthorn) plants. Such treatments also enhanced the antioxidant capacity of the polyphenolic extracts (Kirakosyan *et al.* 2003). *Melissa officinalis* L. was found highly tolerant against water stress. Dry yield under water deficit (0, 12.5, 25.0, 37.5 and 50.0%) varied from 13.05 to 19.20 g/plant. Reduction in yield was not statistically significant till 25% water deficiency, while the EO ratio increased with each increase in water deficiency (Ozturk *et al.* 2004). In one study *Salvia officinalis* L. was subjected to five different watering levels (100, 75, 50, 25 and 0% of ETo) under climatic conditions of extremely high temperature. It was found there were not significant differences among the monitored water rates with regard to the existing relation between the units of dry matter and the EO yields. Water rates affected the composition of *Salvia officinalis* EO, particularly the concentrations of α-pinene, camphene, cineole, α- and β-thujone, bornyl acetate and α-humulene (Corell *et al.* 2009). In other study the endogenous levels of diterpene carnosic acid and α-tocopherol were measured in a drought-recovery cycle in leaves of sage (*Salvia officinalis* L. subs. *officinalis*), a drought-susceptible species, growing in Mediterranean field conditions. As the drought progressed, the carnosic acid amounts in the leaves decreased, giving rise to increased levels of its oxidation products, rosmanol and isorosmanol. At the same time, α-tocopherol levels decreased progressively with drought (Munné-Bosch *et al.* 2001). Drought stress also decreased significantly the foliar fatty acid content in *Salvia officinalis* aerial parts. Besides, moderate water deficit increased the EO yield and the content of main volatile constituents – camphor, α-thujone and 1,8-cineole (Bettaieb *et al.* 2009). The objective of another study was to investigate the effect of drought stress on herbal growth and composition of active constituents in *Salvia miltiorrhiza* Bunge. The results showed that except rosmarinic acid the content of other active constituents increased under water-stress conditions. Water stress significantly increased salvianolic acid B yield and decreased that of tanshinone IIA (Liu *et al.* 2011). Following experiment investigated the influence of soil water stress on plant height, fresh and dry weight and EO content of Iranian *Satureja hortensis* L. The results showed that the accumulation of EO increased significantly under severe water stress at the flowering stage, when the mean leaf water potential decreased from −0.5 to −1.6 MPa. This treatment affected the quantity of the EO more than moderate water stress during the vegetative and flowering stages. From the main oil constituents the amount of carvacrol increased under moderate stress, while γ-terpinene content decreased under moderate and severe water stress treatments (Baher *et al.* 2002). It was found that the plant growth of two lemongrasses [*Cymbopogon nardus.* (L.) Rendle var. *confertiflorus* (Steud.) Bor. and *Cymbopogon pendulus* (Steud.) Wats.] was reduced considerably by drought stress whilst the level of EO was maintained or enhanced. Geraniol and citral, as the major EO constituents, increased substantially in both species (Singh-Sahgwan *et al.* 1994). Drought stress affected growth and EO metabolism in citronella java (*Cymbopogon winterianus*) cultivars. In general, plant growth was reduced considerably while the level of EO was enhanced under water stress. The major oil constituent citronellal decreased significantly in one tested cultivar, while citronellol content, abscisic acid and IAA increased in both the cultivars under drought stress conditions (Shabih *et al.* 2000). Drought stress enhanced alkaloid ajmalicine production in *Catharanthus roseus* (L.) G. Don plants (Abdul Jaleel *et al.* 2008a). In other experiment with two varieties (rosea and alba) of *Catharanthus roseus*, the variations in antioxidant potentials (superoxide dismutase, ascorbate peroxidase, catalase, peroxidase and polyphenol oxidase) and indole alkaloid content were studied, exposed to water deficit stress. The results showed that the antioxidant concentrations and activities of antioxidant enzymes were high under water deficit stress in all parts of the plants. Indole alkaloid content was high in the roots of rosea variety in response to drought stress when compared to alba variety (Abdul Jaleel *et al.* 2008b). In other research the EO content and chemical composition of German chamomile (*Matricaria chamomilla* L.) were determined at different irrigation regimes (irrigation at 100, 85, 70 and 55% of field capacity). Results indicated that the highest amount of EO percent, yield of dry flower and essential oil per pot were obtained from irrigation at 85% of the field capacity and lowest amount of EO percent were obtained when the plants irrigated with 100 and 55% of field capacity (Pirzad *et al.* 2006). Water stress has significant effects on morphological and biochemical characteristics of purple basil (*Ocimum basilicum*). As the soil water content decreased, herbal yield and leaf chlorophyll contents (*a*, *b* and total chlorophyll) decreased, but the amounts of anthocyanin and proline increased (Moeini Alishah *et al.* 2006). Water stress (waterlogging and drought) increased the levels of betulinic acid and phenolic compounds (quercetin, rutin, 1,5-dihydroxyxanthone, isouliginosin B) in *Hypericum brasiliense* Choisy plant (Nacif de Abreu *et al.* 2005). The objective of another study was to determine the contents of secondary metabolic substances such as resveratrol, SOD, phenolic compounds and free amino acids in *Rehmannia glutinosa* under temperature and water stress. Of the 16 individual phenolic compounds, myricetin showed the highest concentrations in the water deficiency (−1.18 MPa) and control (−1.04 MPa) and low temperature (15°C) treatment. The 21 free amino acids in *R. glutinosa* decreased at high temperature and with a water deficiency (Chung *et al.* 2006). In the following study, the changes in the alkaloid content (berberine, jatrorrhizine and palmatine) in Amur corktree (*Phellodendron amurense*) under different water conditions (mild drought, severe drought, waterlogging and control) were discussed. It was shown that mild drought was generally beneficial to the synthesis and accumulation of the above-mentioned alkaloid contents. The three alkaloid contents did not show great changes under severe drought, whereas those contents had significantly reduced under waterlogging compared with controls (Xia *et al.* 2007). An experiment was carried out to determine the association of tea polyphenols with water stress and their suitability as indicators for drought tolerance. The experiment was conducted in a 'rain-out' shelter, and consisted of six tea clones and four levels of soil water contents (38, 30, 22, and 14% v/v). The results indicate that declining soil water content reduced both growth and content of polyphenols in tea (Cheruiyot *et al.* 2007). Water stress increased the yield of EO from the leaves of plain-leafed and curly-leafed cultivars of parsley, but not turnip-rooted cultivar. Water stress also caused changes in the constituents of EO (principally 1,3,8-p-menthatriene, myristicin, terpinolene + p-cymenene), but these changes varied between cultivars (Petropoulos *et al.* 2008). Water deficit induced a significant reduction in growth parameters and fatty acid content and an increase in the EO oil compounds (carvone and limonene) in caraway (*Carum carvi* L.) (Laribi *et al.* 2009).

CONCLUSION

It can be concluded that in soilless greenhouse production the productivity and quality of lemon catmint (*Nepeta cataria* L. *f. citriodora*), lemon balm (*Melissa officinalis* L.) and sage (*Salvia officinalis* L.) mainly depended on drought stress. Herbal yield, amount and composition of essential oils and polyphenols significantly depended on substrate moisture, while antioxidative capacity of polyphenol-rich extracts was affected to a lesser extent.

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