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

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ABSTRACT

Various environmental and growing factors can affect not only the primary, but also the secondary metabolism in herbs. Nitrogen stress considered to be one of the main environmental factors influencing the plant growth and quality. This study investigated the impact of different nitrogen concentrations in nutrient solution (50, 150 and 300 mg/l) 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.) under soilless greenhouse cultivation. It was found that high nitrogen concentration of 300 mg/l was effective for herbal yield of lemon catmint. By contrast, low nitrogen concentration of 50 mg/l was positive for herbal productivity of sage, while for lemon balm both 50 and 150 mg/l concentrations provided maximum results. Nitrogen stress had an impact on essential oil content of lemon balm and sage, but not of lemon catmint. In case of lemon catmint high nitrogen concentration of 300 mg/l was favourable for maximum yield of essential oil, while for sage medium concentration of 150 mg/l was the best and for lemon balm there was no significant difference between the treatments. The essential oil composition of selected medicinal and aromatic plants was mainly affected by tested nitrogen concentrations. The influence of nitrogen stress was also significant for content and composition of polyphenols in studied herbs, as well as for antioxidative capacity of polyphenol-rich extracts by the ABTS system.

Keywords: bioactive compounds, hydroponics, *Melissa officinalis*, *Nepeta cataria f. citriodora*, nitrogen concentration, *Salvia officinalis*

INTRODUCTION

Environmental and growing factors can influence the yield and biochemistry of herbs. Besides climatic conditions at the growing site, others are edaphic factors, such as nitrogen stress, which are of particular importance as natural tools for improvement or limiting productivity and quality of plants. Some reports already described the impact of nutrition on the herbal yield and content of bioactive compounds of medicinal and aromatic plants (Ruminska 1978a; Franz 1983; Ratnayaka *et al.* 1998; Baricevic *et al.* 1999; Martinetti *et al.* 2006; Aziz *et al.* 2008; Said-Al Ahl *et al.* 2009; Colling *et al.* 2010; Matos *et al.* 2010). Though, for such crops, the yield of secondary plant substances is predominant, little is known yet on secondary metabolism as influenced by nitrogen concentration in nutrient solution under soilless greenhouse conditions. The aim of this study is to investigate the effect of nitrogen 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. Having such information on the effects of different nitrogen concentrations, the herbal yield, quantity and quality of valuable pharmaceutical substances in plant material can be managed under protected soilless cultivation.

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 greenhouse. 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. 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 3 ebb- and flood tables were used with 20.0 m^2 surface area for each with 9 plants per m^2 (45 plants per treatment with 4 repetitions). The plants were automatically fed twice a day for the duration of 60 minutes. The nutrient solution was made up from 70g "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 50, 150 or 300 mg/l nitrogen (composed of ammonium nitrate (Merck) and calcium nitrate (Merck)) with pH of 5.5-7.3 and EC of 1.5-2.6, 1.8-5.4 and 2.3-7.4 mS/cm, respectively. 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 g/plant 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

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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 × 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 productivity of herbs was mainly affected by the level of nitrogen in nutrient solution under soilless growing conditions. It was found hat high nitrogen concentration of 300 mg/l was favorable for herbal yield of lemon catmint, while low and medium concentrations (50 and 150 mg/l) were optimal for high productivity of lemon balm. In the case of sage comparatively low nitrogen concentration of 50 mg/l provided maximum results. In the case of lemon catmint and sage 50 mg/l nitrogen concentration resulted a lower percent of dry weight (**Table 1**).

Table 1 Influence of nitrogen concentration on herbal yield.

Species	Nitrogen concentration, mg/l	Fresh medicinal material, g/plant	Raw medicinal material	
			g/plant	% (dry weight)
Lemon catmint	50	238.6 b	35.4 c	14.8 b
	150	253.8 b	46.8 b	18.4 a
	300	289.2 a	52.2 a	18.0 a
LSD_{05}*	---	34.7	5.3	2.4
Lemon balm	50	125.9 a	22.9 a	20.0 a
	150	110.6 a	22.1 a	20.0 a
	300	84.4 b	17.5 b	20.0 a
LSD_{05}*	---	16.4	2.8	2.6
Sage	50	156.6 a	21.1 a	13.5 b
	150	102.4 b	17.0 b	16.6 a
	300	80.7 c	14.2 c	17.6 a
LSD_{05}*	---	18.8	2.5	2.3

* Within the same species

Different nitrogen concentrations have a considerable influence on EO content of lemon balm and sage, but not of lemon catmint. The treatments with comparatively high nitrogen availability in nutrient solution (150 and 300 mg/l) provided maximum content of EO in lemon balm, while for sage 150 mg/l was optimal (**Fig. 1**). Calculations for EO yield showed that high nitrogen stress (300 mg/l) provided maximum yield in the case of lemon catmint, while in lemon balm there were no significant differences between the treatments. In the case of sage, 150 mg/l nitrogen concentration provided maximum yield of EO (**Fig. 2**).

EO composition of lemon catmint depended on tested nitrogen levels (**Table 2**). The identified main substances of lemon catmint EO were geraniol (27.35-30.49%), geranial (15.59-18.85%), nerol (14.19-17.98%), neral (12.04-14.64%) and citronellol (11.19-13.98%). Twenty-two identified compounds represented approximately 97% of the total oil. Different nitrogen concentrations had a considerable influence on the content of a number of compounds in lemon catmint EO. The increase of nitrogen concentration in nutrient solution from 50 up to 300 mg/l decreased the content of some main compounds, such as neral and geranial, while in case of citronellol and nerol it was on the contrary. The content of geraniol was not essentially affected by nitrogen concentration in nutrient solution. It was also notable that the content of phytol was more than 10 times

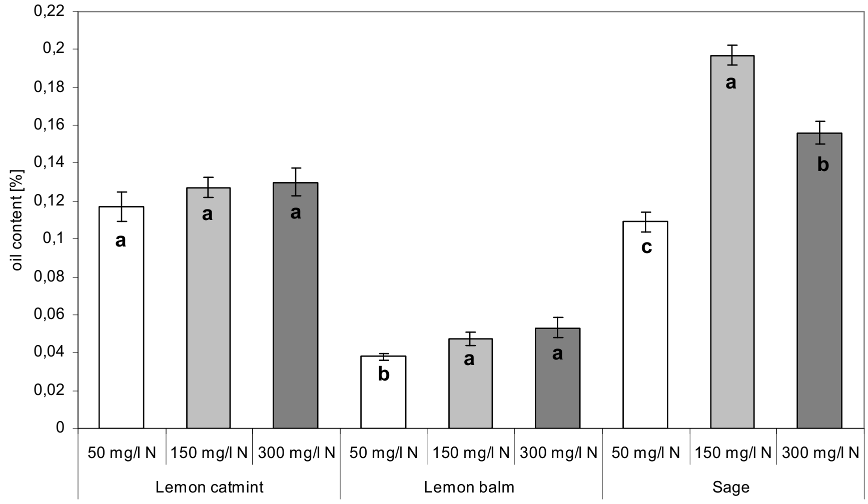

Fig. 1 Influence of nitrogen concentration on essential oil content of herbs. Values represent mean ± standard deviation (SD). Statistics are made within the same species.

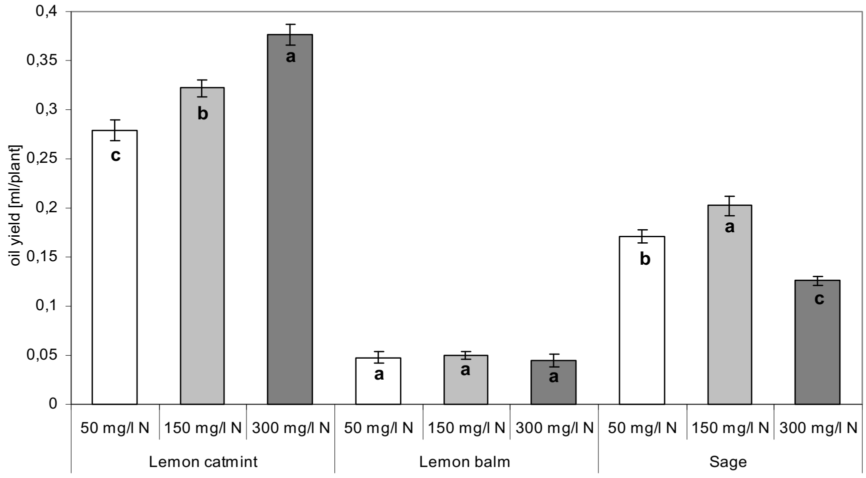

Fig. 2 Influence of nitrogen concentration on essential oil yield of herbs. Values represent mean ± standard deviation (SD). Statistics are made within the same species.

Table 2 Influence of nitrogen concentration on lemon catmint essential oil composition.

Compound*	Nitrogen concentration, mg/l		
	50	150	300
	Content in essential oil, %		
cis-ocimene	0.12 b**	0.12 b	0.14 a
cis-3-hexenal	0.02 a	0.01 b	0.02 a
trans-ocimene	0.01 a	0.01 a	0.01 a
cis-3-hexenyl acetate	0.02 a	0.02 a	0.02 a
6-methyl-5-hepten-2-on	1.32 a	1.19 a	1.32 a
trans-3-hexen-1-ol	0.06 b	0.07 a	0.05 c
cis-3-hexen-1-ol	2.20 b	1.93 b	3.06 a
trans-2-hexen-1-ol	0.41 a	0.22 c	0.28 b
citronellal	1.73 a	1.89 a	1.93 a
linalool	0.11 a	0.10 a	0.12 a
β-caryophyllene	1.08 b	1.02 b	1.25 a
citronellyl acetate	0.04 a	0.02 b	0.04 a
α-humulene	0.08 b	0.08 b	0.10 a
neral	14.64 a	13.23 ab	12.04 b
neryl acetate	0.04 b	0.05 a	0.04 b
geranial	18.85 a	17.35 ab	15.59 b
geranyl acetate	0.15 a	0.17 a	0.12 b
citronellol	11.19 b	12.98 a	13.98 a
nerol	14.19 b	17.04 a	17.98 a
geraniol	30.49 a	29.88 a	27.35 a
β-ionone	0.02 a	0.02 a	0.02 a
phytol	0.15 b	0.11 b	1.55 a

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

Table 3 Influence of nitrogen concentration on lemon balm essential oil composition.

Compound*	Nitrogen concentration, mg/l		
	50	150	300
	Content in essential oil, %		
β-myrcene	0.06 b**	0.02 c	0.08 a
cis-ocimene	0.02 c	0.12 a	0.09 b
cis-3-hexenal	0.04 a	0.04 a	0.03 b
trans-ocimene	0.44 a	0.36 b	0.28 c
6-methyl-5-hepten-2-on	0.87 c	1.25 b	1.48 a
trans-3-hexen-1-ol	0.83 c	1.35 a	1.01 b
trans-2-hexen-1-ol	0.27 b	0.34 a	0.26 b
1-octen-3-ol	0.23 b	0.29 a	0.26 ab
citronellal	1.60 a	0.87 b	0.82 b
linalool	0.51 b	0.59 a	0.48 b
not identified	0.53 b	0.55 ab	0.62 a
not identified	0.98 b	1.01 ab	1.13 a
β-caryophyllene	1.33 a	1.04 b	1.25 a
α-humulene	0.07 a	0.05 c	0.06 b
neral	26.86 a	28.28 a	28.63 a
neryl acetate	0.23 b	0.22 b	0.29 a
geranial	35.99 a	37.01 a	37.92 a
geranyl acetate	2.58 ab	2.25 b	2.79 a
citronellol	1.37 a	1.04 b	0.72 c
nerol	4.89 a	4.05 b	3.65 b
geraniol	18.36 a	17.67 a	16.26 a
cis-9-octadecenoic acid methyl ester	0.07 b	0.09 a	0.09 a

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

higher at high nitrogen stress (300 mg/l).

EO of lemon balm composed mostly of geranial (35.99-37.92%), neral (26.86-28.63%) and geraniol (16.26-18.36%) under soilless greenhouse conditions (**Table 3**). Twenty identified compounds represented about 97, 97 and 96% of the total oil at 50, 150 and 300 mg/l nitrogen concentrations, respectively. Except of three major constitutions, the contents of all other identified compounds were affected by nitrogen concentrations in nutrient solution.

Among the twenty-five identified compounds in sage EO, α-thujone (26.44-48.08%), camphor (12.37-20.02%), β-thujone (6.03-15.65%) and 1,8-cineole (7.67-11.03%) were the major ones (**Table 4**). Identified compounds represented about 97, 97 and 98% of the total oil at 50, 150 and 300 mg/l nitrogen, respectively. Different nitrogen concentrations had a considerable influence on content of all identified compounds in sage EO. The increase of nitrogen concentration from 50 up to 150 mg/l decreased the content of α-thujone, but a further increase up to 300 mg/l produced maximum amount of this compound (48.08%). In case of camphor, the increase of nitrogen concentration influenced negatively the content of this substance in sage oil. The contents of β-thujone and 1,8-cineole peaked up at 150 mg/l.

The influence of tested nitrogen concentrations on the content of polyphenols in studied herbs was also significant (**Table 5**). The increase of nitrogen concentrations in nutrient solution maximized the content of flavonoids in all three species. At the same time the changes in the polyphenolic content were more essential for lemon balm and the difference between the minimum and maximum results was more than 100%.

HPLC analysis of polyphenols showed differences in sensitivity of main polyphenols to nitrogen stress as well. In lemon catmint, three major identified polyphenolic compounds (caffeic, rosmarinic and p-coumaric acids) enriched with highest nitrogen concentration (300 mg/l) (**Fig. 3**). In lemon balm, rosmarinic acid (peak 2) reached its maximum at 50 mg/l, while caffeic acid (peak 1) and melitric acid (peak 3) showed no essential differences (**Fig. 4**). In sage, rosmarinic acid (peak 2) was maximal at high nitrogen stress treatment, while the high concentration of caffeic acid (peak 1) was recorded at 150 mg/l. Melitric acid (peak 3)

Table 4 Influence of nitrogen concentration on sage essential oil composition.

Compound*	Nitrogen concentration, mg/l		
	50	150	300
	Content in essential oil, %		
α-pinene	2.46 b**	2.80 a	2.41 b
camphene	2.31 b	2.92 a	1.72 c
β-pinene	3.06 b	3.34 b	3.87 a
sabinene	0.32 a	0.28 b	0.32 a
β-myrcene	0.79 b	0.94 a	0.93 a
α-terpinene	0.12 b	0.15 a	0.17 a
limonene	1.00 a	1.01 a	0.78 b
1,8-cineole	8.16 b	11.03 a	7.67 b
cis-ocimene	0.16 a	0.13 b	0.17 a
cis-3-hexenal	0.11 b	0.17 a	0.10 b
γ-terpinene	0.28 b	0.35 a	0.39 a
p-cymole	0.07 b	0.08 ab	0.09 a
terpinolene	0.28 a	0.27 a	0.23 b
trans-3-hexen-1-ol	0.71 a	0.57 b	0.62 b
trans-2-hexen-1-ol	0.15 a	0.13 b	0.13 b
α-thujone	34.14 b	26.44 c	48.08 a
β-thujone	6.03 b	15.65 a	6.05 b
camphor	20.02 a	17.50 b	12.37 c
bornyl acetate	0.89 a	0.61 b	0.32 c
β-caryophyllene	2.23 a	1.47 c	1.93 b
α-humulene	4.07 a	3.74 a	2.86 b
borneol	1.71 a	1.34 b	1.14 b
myrtenol	0.16 a	0.07 b	0.08 b
viridiflorol	4.52 a	4.30 a	3.50 b
manool	3.08 a	1.89 b	1.57 b

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

Table 5 Influence of nitrogen concentration on polyphenol content of herbs.

Species	Nitrogen concentration, mg/l	Gallic acid equivalents-GAE [mM]
Lemon catmint	50	0.65 ± 0.02 b
	150	0.68 ± 0.02 b
	300	0.78 ± 0.03 a
LSD_{05}*	---	0.08
Lemon balm	50	0.83 ± 0.03 c
	150	1.35 ± 0.04 b
	300	1.85 ± 0.05 a
LSD_{05}*	---	0.25
Sage	50	1.48 ± 0.04 b
	150	1.80 ± 0.05 a
	300	1.93 ± 0.05 a
LSD_{05}*	---	0.29

* Within the same species

showed no essential differences in this context and was detectable only in very small amounts (**Fig. 5**).

A higher antioxidative capacity of polyphenol-rich extracts of lemon balm and sage was noted even in 10 fold lower concentration (1:200) (**Fig. 7, 8**) compared to lemon catmint at 1: 20 (**Fig. 6**). In case of lemon catmint, the polyphenolic extracts obtained from 50 mg/l nitrogen treatment showed a little bit higher antioxidative capacity by the ABTS-system (**Fig. 6**). Polyphenolic extracts of lemon balm and sage grown under different nitrogen levels showed correlations between their antioxidative capacity and content of rosmarinic acid. The maximum concentration of rosmarinic acid at 50 mg/l in lemon balm (**Fig. 4**) and at 300 mg/l in sage (**Fig. 5**) provided accordingly high antioxidative capacity by the ABTS-system. Additionally, the shapes of the curves differ considerably with a comparatively longer lag phase for 50 mg/l treatment in lemon balm (**Fig. 7**) and for 300 mg/l treatment in sage (**Fig. 8**).

DISCUSSION

This study confirms that nitrogen stress is an important external factor that influences the productivity and quality of selected medicinal and aromatic plants under soilless growing conditions. Herbal yield, content and composition of EO were mostly affected by different nitrogen concentration in nutrient solution. The analysis showed some direct correlations between the nitrogen level in fertilizer and the amount of some volatile substances in herbs. In particular, the increase of nitrogen concentration in nutrient solution from 50 up to 300 mg/l increased the content of 6-methyl-5-hepten-2-on (in lemon balm) and decreased the content of trans-ocimene, citronellol (in lemon balm) and camphor, bornyl acetate (in sage). The tested nitrogen concentrations had a significant effect on content and composition of polyphenols, as well as on antioxidative capacity of flavonoid-rich extracts by the ABTS-system.

In spite of growing interest during recent years for natural ways to produce high quality herbal material with valuable pharmaceutical compounds without any genetic manipulation, the studies on the effects of nitrogen stress on herbal yield and production of bioactive compounds under controlled soilless conditions are still comparatively seldom. Several authors have published data on the effect on nitrogen concentration in nutrient solution and nitrogen stress on herbal yield, content and composition of bioactive substances of medicinal and aromatic plants under soil and soilless conditions. In one study it was reported that there was no remarkable influence of levels, forms and time of adding nitrogen fertilizer on the total alkaloid content of *Datura innoxia* Mill. (Ruminska *et al.* 1978b). In other field experiment, it was found that the EO content of the peppermint (*Mentha piperita* L.) and sweet basil (*Ocimum basilicum* L.) increased proportionally with N-supply, but it changed according to the optimal curve in the green dill (*Anethum graveolens* L.) (Hornok 1983). Nitrogen dressing increased biomass and EO yield of peppermint (*Mentha x piperita* L.)

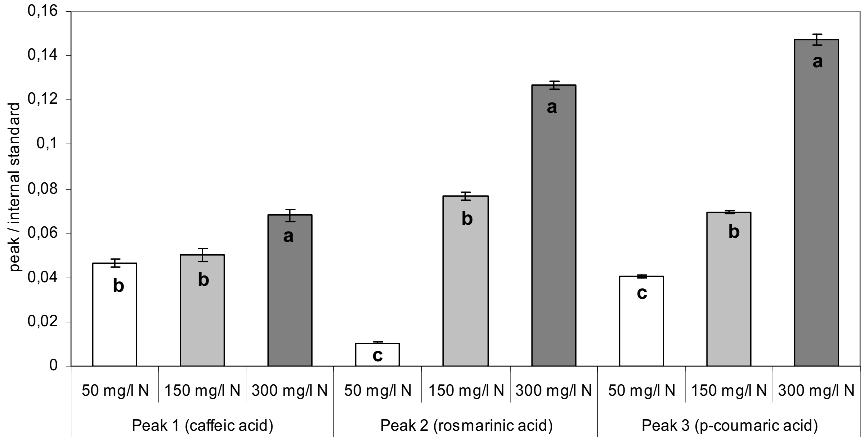

Fig. 3 Influence of nitrogen concentration on quantity of major polyphenols in lemon catmint. Values represent mean ± standard deviation (SD). Statistics are made within the same compound.

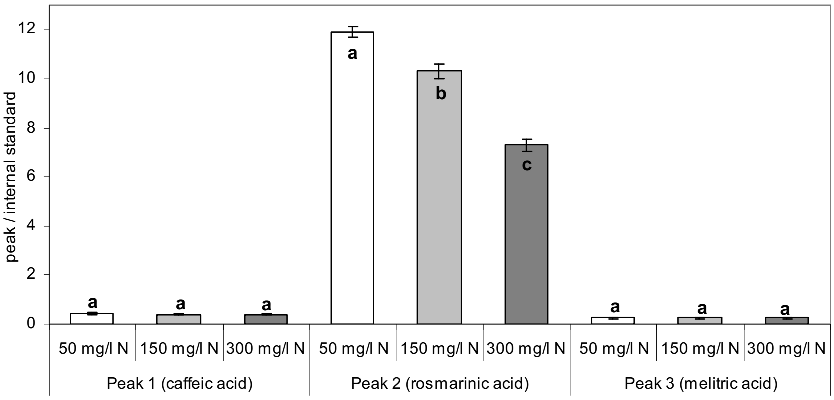

Fig. 4 Influence of nitrogen concentration on quantity of major polyphenols in lemon balm. Values represent mean ± standard deviation (SD). Statistics are made within the same compound.

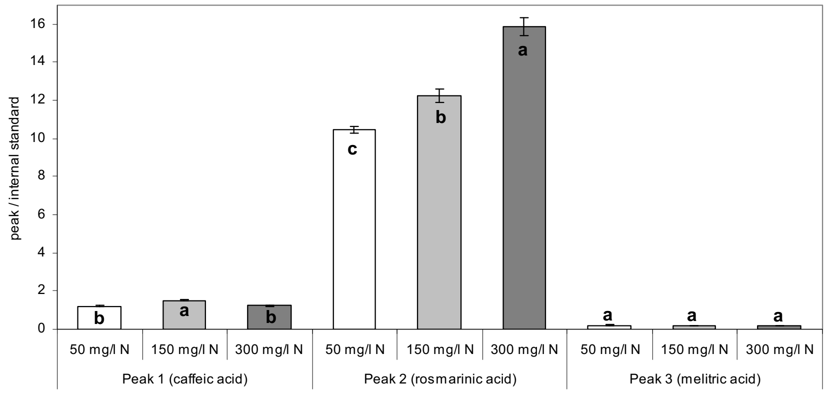

Fig. 5 Influence of nitrogen concentration on quantity of major polyphenols in sage. Values represent mean ± standard deviation (SD). Statistics are made within the same compound.

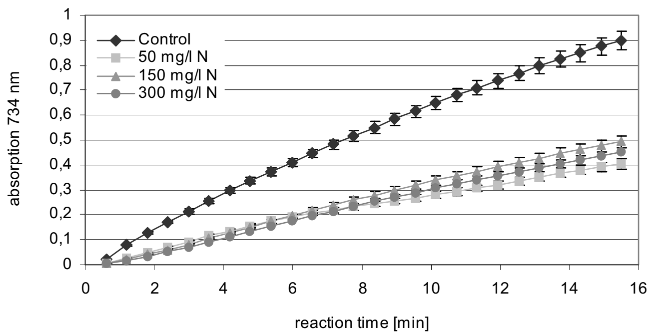

Fig. 6 Influence of nitrogen concentration on antioxidative capacity of polyphenol-rich extracts of lemon catmint.

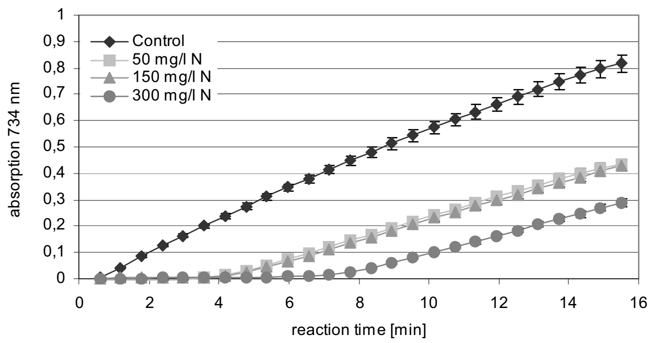

Fig. 8 Influence of nitrogen concentration on antioxidative capacity of polyphenol-rich extracts of sage.

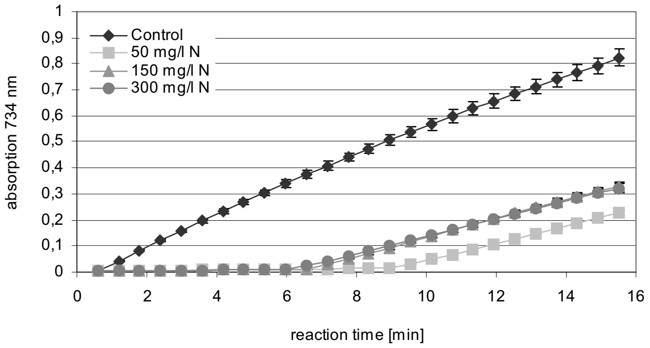

Fig. 7 Influence of nitrogen concentration on antioxidative capacity of polyphenol-rich extracts of lemon balm.

cultivar "Italo-Mitcham", but decreased the percentage of leaves over biomass (Piccaglia *et al.* 1993). Another study found that N rates increase thyme (*Thymus vulgaris* L.) crop, but differences in the yield of EO were not remarkable (Baranauskienè *et al.* 2003). Other research reported on the effect of N fertilization on growth and EO of *Eucalyptus torquata* and *Eucalyptus angulosa*. It was found that the two levels of N (200 and 400 kg/fed) greatly increased the percentage of EO of both species. N at 200 kg/fed gave the highest percentage of cineole. Whereas, N at 400 kg/fed, increased the percentage of citronellol of *Eucalyptus torquata* and *Eucalyptus angulosa* oils (Mahdi *et al.* 1987). High levels of N promoted increase in leaves weight, but less oil content with low menthol content in *Mentha arvensis* L. (Nilson *et al.* 2001). Other study reported on the effect of different nitrogen concentration (100, 150 and 200 mg/l) in the nutrient solution on the volatile constituents of leaves of *Salvia fruticosa* Mill. in NFT. It was found that the constituents of the EO showed qualitative and quantitative differences due to the sampling at different periods as well as different concentrations of nitrogen in the nutrient solutions. The effect of nitrogen concentration was minor with a remarkable decrease to 200 mg of N/l at the end of the seed formation stage (Karioti *et al.* 2003). The decrease in EO content at 200 mg/l agrees with the results on *Mentha arvensis* L. sand culture. It was shown that the EO content increased with an increase in nitrate-N (NO_3-N) up to 16 mequiv/L, beyond which it decreased (Singh *et al.* 1989). Other study found no effect of NO_3-N level at similar concentrations (100, 150, and 200 mg/l) on the EO content in leaves and bracts of *Origanum dictamnus* L. grown with NFT (Economakis *et al.* 1999). The herbal yield of hydroponically grown basil (*Ocimum basilicum* L.) was improved by increasing the nutrient solution nitrogen concentration up to 300 mg/l. EO yield did not show any significant changes related to treatment variables and was between 0.2-0.5% (v/w). Nitrogen supply did not have a marked effect on oil composition (Smith *et al.* 1997). In other experiment, the EO content of fennel (*Foeniculum vulgare* Mill.) was multiplied by increasing N-portion. The highest EO content (7.25 g/100 g) was at 200 kg/ha N-portion. The increase of was 81.25% compared to the control (Omidbaigi *et al.* 1992). The yield contributing characters, drug and straw yields, content of the EO and flavonoids of different camomile genotypes significantly affected by the sowing seasons and favoured by increased nitrogen level (Letchamo 1992). Another study investigated the productivity of asparagus (*Asparagus officinalis* L.) in aeroponics and water culture. It was found out that 100 mg/l nitrogen level in nutrient solution (Flory № 1) is more acceptable for getting maximum yield with a high content of available carbohydrates in plant roots (Manukyan *et al.* 2005). The effect of N supply on the production of hypericins (hypericin and pseudohypericin) in leaves of St. John's wort (*Hypericum perforatum* L.) was examined using plants grown in sand culture and soil. In sand culture, 56 days with decreased N levels resulted in increased production of hypericins in leaves. A short-term low N stress in sand culture also resulted in increased production of leaf hypericins. Growth in a low N-containing soil resulted in elevated levels of hypericins; such production was decreased by supplementation of the soil with additional N. Increased production of hypericins in leaves did not require the N supply to be decreased to levels that resulted in N deficiency symptoms. Moreover, alteration in the production of leaf hypericins occurring with changes in N supply did not alter the concentration ratio of pseudohypericin and hypericin (Briskin *et al.* 2000). Other research studied the feasibility of camptothecin (CPT) production from *Camptotheca acuminata* in hydroponic culture. High concentrations of CPT were found in leaves of plants in hydroponic culture, but the N concentration in the culture medium did not affect tissue CPT concentration, plant weight, plant height, leaf number, lamina dry weight per area, total stem weight, total leaf weight, or total leaf CPT yield (Li *et al.* 2005). In following study the effect of nitrogen and phosphorus supply was investigated on 3-year-old garden thyme (*Thymus vulgaris* L.) population. After the application of six different dosages of nitrogen (0, 100, 150, 200, 250, and 300 kg/ha) and phosphorus (0, 50, 100, 150, 200, and 250 kg/ha), it was determined that N and P fertilization had a significant effect on herb yield and EO content but did not change the thymol content (Omidbaigi *et al.* 2002). The activities of antioxidant enzymes viz. glutathione reductase, GR; superoxide dismutase, SOD; peroxidase, POD; catalase, CAT and glutathione-*S*-transferase, GST and alkaloid accumulation were investigated in leaf pairs (apical, middle, basal) and in roots of *Catharanthus roseus* (Madagascar periwinkle) seedlings under the conditions of different nitrogen sources (20 mM KNO_3 and 2 mM NH_4Cl) and salinity. Changes in antioxidant enzyme activity caused by different nitrogen sources differed in all leaf pairs, as well as in roots of *C. roseus*. Ammonium-fed plants showed higher CAT, GR and GST activity in leaf pairs as well as in roots, while POD and SOD activity were higher in nitrate-fed plants. Higher peroxidase activity concomitant with the increased accumulation of alkaloid was found in all leaf pairs, as well as in roots of *C. roseus* of NO_3^- fed plants as compared to NH_4^+ fed content (Misra *et al.* 2006). In other experiment with periwinkle, the effect of N fertilization on the content of anti-cancer and anti-hypertension alkaloids of two high alkaloid content mutants of periwinkle was studied, in comparison with their parental variety, to determine the possibility of further increasing their alkaloid contents. The three genotypes were evaluated at three levels of N (0, 100 and 150 kg/ha) fertilization in a split plot experiment. N fertilization significantly increased the content of alkaloids both in leaves and roots of all genotypes. Over genotypes, application of 150 kg/ha of N resulted in an increase of 42 and 32% in the content of leaf and root alkaloids, respectively. However, the increase was highest in genotypes with the lowest content of leaf or root alkaloids. The high alkaloid mutants grown at 150 kg ha^{-1} of N fertilization exhibited an increase of 87 and 56% in the content of leaf and root alkaloids, respectively, when compared with their parental variety grown without nitrogen fertilization (Sreevalli *et al.* 2004). In order to investigate the effect of different levels of nitrogen and potassium in Johnson solution on EO content of peppermint (*Mentha piperita*) in hydroponics, an experiment was carried out were peppermint plants grown in sand media under open-air soilless culture condition and the system was bag culture method. The treatments included N in five levels (105, 135, 165, 195 and 225 ppm) and K in three levels (138, 178 and 218 ppm). The results showed that the highest EO production was in N at 195 ppm and K at 218 ppm concentration (Mollafilabi *et al.* 2010). In another study, the effects of long-term nitrogen deficiency (N 0.1 mM for 4 months) on growth, phenolic content and activity of phenylalanine ammonia lyase (PAL; EC 4.3.1.5) were investigated in the leaves, inflorescences and roots of yarrow (*Achillea collina* Becker ex Rchb.) grown in hydroponics. Nitrogen starvation decreased plant growth and the leaves' total nitrogen, amino acids, proteins, chlorophylls and carotenoids contents indicating that the primary metabolism was severely limited by low nitrogen availability. The amount of total phenolics and the antioxidant capacity were higher in leaves and roots of nitrogen-starved compared to control plants. Nitrogen starvation significantly increased the contents of phenolic acids (5-*O*-caffeoylquinic acid (chlorogenic acid) and 3,5 and 4,5-di-*O*-caffeoylquinic acids) and the PAL activity in leaf and root tissues (Giorgi *et al.* 2009). The following study aimed to examine the effects of nitrogen fertilization on the growth performance, betaine and polysaccharide concentrations in different plant parts of *Lycium barbarum*. *L. barbarum* seedlings were subjected to organic (Org 1: 15 g N/pot; Org 2: 30 g N/pot) and chemical (Chem 1: 2.5 g N/pot; Chem 2: 5 g N/pot) N fertilizer treatments. Results showed that the yield and uptake of N in plants of Org 2 (high organic fertilizer) treatment were the highest. Although plants of Chem 2 (high chemical fertilizer) treatment demonstrated to contain the highest N concentration, its yield in biomass was the lowest. A decrease in soluble sugar, polysaccharide and betaine concentrations was noted with increasing application of chemical N fertilizer. However, an increase in the organic N fertilizer favors the betaine synthesis (Chung *et al.* 2010). The next study investigated the interactive effects of light intensity and controlled-release nitrogen fertilizer (CRNF) supply on growth, gas exchange, and chlorophyll (Chl) fluorescence parameters of two species of potted *Hosta* seedlings (*Hosta clausa* var. *ensata* and *Hosta ventricosa*). N_4 (4 g of CRNF per pot), N_8 (8 g of CRNF per pot) and sometimes N_{12} (12 g of CRNF per pot) significantly increased total dry weights, net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), the maximum quantum yield of PSII photochemistry (F_v/F_m), the maximum ratio of quantum yields of photochemical and concurrent non-photochemical processes in PSII (F_v/F_0), actual efficiency of photochemical energy conversion in PSII under light (Φ_{PSII}), and photochemical quenching coefficient (qP), but significantly decreased internal CO_2 concentration (C_i) and nonphotochemical Chl fluorescence quenching (NPQ) compared to control plants at different growth stages of the two *Hosta* species in two levels of light intensities (50% of natural light (L_{50}) and 70% of natural light (L_{70})) (Zhang *et al.* 2011).

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.) significantly depended on nitrogen stress. Herbal yield, content and composition of essential oils and polyphenols, as well as the antioxidative capacity of polyphenol-rich extracts were mainly affected by nitrogen concentration in nutrient solution.

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