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Improvement of the quality in hydroponically grown fresh aromatic herbs by inducing mild salinity stress is species-specific

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ABSTRACT

Profitable hydroponic production requires high quality fresh water, which is often not available for agricultural use, while desalinisation of salty water is an expensive and unsustainable technology. In the present study, we assessed the effect of mild salinity stress during the soilless cultivation of fresh peppermint and spearmint in the floating system on biomass yield, produce quality and plant secondary metabolite content. Peppermint and spearmint plants were grown for 25 days on a nutrient solution (NS) supplemented with three different NaCl concentrations (0 mM, 10 mM or 20 mM NaCl). The plant height, root length, fresh and dry weight were recorded and composition was determined on fresh tissue. The composition of essential oil was determined upon hydrodistillation and that of polyphenolic compounds by targeted ultra-performance liquid chromatography coupled with mass spectrometer (UPLC-MS/MS). Plant growth was not suspended by the addition of NaCl in the NS, except for the plant height at the highest salinity level. In peppermint, the nutritional composition was not affected by the salinity, whereas it was significantly improved in spearmint as confirmed by the nitrate content decrease and the total antioxidant capacity, total soluble phenol, total carotenoid and essential oil content increases. Simultaneously, no effect of the salinity on essential oil or polyphenolic composition in both plants was induced. In conclusion, peppermint and spearmint production is feasible in the floating system even under mild salinity conditions, without negatively affecting either the crop yield or the plant's essential oil or phenolic composition. Indeed, low salinity levels improved the nutritional composition of spearmint plants.

Keywords: abiotic stress, antioxidants, essential oil, polyphenols, sodium chloride, soilless production

Abbreviations: AEAC, ascorbic acid equivalents; $CaCl_2$, calcium chloride; CO_2, carbon dioxide; EC, electrical conductivity; GAE, gallic acid equivalents; GC-MS, Gas Chromatography-Mass Spectroscopy; LSD, least significant differences; NaCl, sodium chloride; NS, nutrient solution; UPLC-MS/MS, ultra-performance liquid chromatography-tandem mass spectrometry.

INTRODUCTION

Soil salinisation is one of the detrimental effects of climate change, and it is often combined with low quality water during irrigation and poor soil drainage, especially in semi-arid regions all over the world

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(Arslan et al., 2018; Rezaie et al., 2019). Moreover, fresh groundwater is slowly converted into saline water and thus rendered unsuitable for agricultural use, due to excessive pumping for irrigation use followed by seawater fusion, waterlogging and nutrient leaching (Alaghmand et al., 2013; Pulido-Bosch et al., 2018). These factors have triggered growers' willingness to shift towards hydroponic vegetable production (Niu et al., 2018), which however requires the use of optimum quality water that is unfortunately not always available for agricultural activities (Niu et al., 2018; Yousefi et al., 2020). Deterioration of groundwater quality often imposes the need for establishment of desalinisation water treatment technologies, such as the reverse osmosis technique, which not only are substantially expensive but also simultaneously convert only part of the source water into water suitable for irrigation and reject the remaining, which typically amounts to around 60%, due to extreme salinity loading (Arslan et al., 2018; Niu et al., 2018; Pulido-Bosch et al., 2018; Atzori et al., 2019).

Using low quality water with high electrical conductivity (EC) carrying a large amount of salts such as sodium chloride (NaCl) causes the absorption and over-accumulation of sodium and chloride ions in tissues, which subsequently induce severe salt toxicity, tissue dehydration, nutrient imbalance and oxidative stress (Munns, 1993; Lazof et al., 1998). On the other hand, it has been demonstrated that modification of the EC in the nutrient solution (NS) has efficiently improved the quality of some vegetables, and such a practice has already been recommended for use in agricultural production (Rouphael et al., 2012). Furthermore, salinity stress may even induce secondary metabolism in plants and, consequently, the accumulation of human health promoting secondary metabolites, such as terpenes and polyphenolic compounds (Tarchoune et al., 2013; Tounekti et al., 2011; Verma and Shukla, 2015; Thakur et al., 2019).

The floating tray system exhibits many advantages in comparison to other hydroponic systems, such as low installation cost, simplicity and functionality of operation, high water and nutrient use efficiency, accelerated cultivation cycles and eventually total absence of soil residues from the harvested produce (Rodríguez-Hidalgo et al., 2010). Several aromatic plants of the Lamiaceae family are suitable to be grown in this hydroponic system, given that there is a significant demand for a high volume of water in order to increase crop yield (Peter, 2001). Peppermint (*Mentha* × *piperita* L.) and spearmint (*Mentha spicata* L.) are two perennial herbs of high economic value, from which a combination of uses can be obtained, including in cooking, phytomedicine and food industry (Charles, 2013). They are consumed fresh or dry and are rich in phytonutrients, such as vitamins, minerals and natural antioxidants (Peter, 2001; Charles, 2013).

Spearmint cultivation using the deep flow technique has already been successfully tested (Vimolmangkang et al., 2010; Chrysargyris et al., 2017); however, there is no available literature relevant to peppermint production. Moreover, the response of these two species to salinity has only been studied under extreme treatments with high salt concentration.

Therefore, the aim of this study was to investigate the feasibility of a commercial soilless production of peppermint and spearmint on the floating trays system, as well as to assess the possibility of adapting mild salinity conditions in the NS of a non-recirculating closed hydroponic system as a tool to improve the quality and nutritional value of fresh peppermint and spearmint plants.

MATERIALS AND METHODS

Hydroponic system

A floating hydroponic system was established in a glasshouse at the Aristotle University of Thessaloniki farm (Thermi, Greece) during April–May 2019. Nine plastic basins (70 cm × 65 cm × 20 cm, L × W × H) were filled with 50 L of NS each (Table 1) and were adjusted to three salinity levels with the addition of 0 mM, 10 mM or 20 mM NaCl. Three replicates were used per salinity level with one basin per replicate. During the cultivation, the mean temperature inside the greenhouse ranged between 19.4 °C and 29.9 °C and the mean relative humidity between 49.3% and 71.4%.

Plant material and treatments

Peppermint and spearmint rooted grafts were collected from two local populations preserved in the Department of Medicinal and Aromatic Plants of the Institute of Plant Breeding and Genetic Resources (Thermi, Greece). The grafts were transplanted at a density of 230 plants per square metre on expanded polystyrene trays filled with a commercial enriched peat substrate. The peat substrate was Klasmann Potgrond H with the following composition and properties: 70% frozen through black peat and 30% white peat, fine structure (0–8 mm), fertiliser level = 1.5 g · L^{-1}, 210 mg · L^{-1} N,

Table 1. The composition of the NS used in the floating tray system for peppermint and spearmint cultivation.

Macronutrients (mg · L^{-1})		Micronutrients (µg · L^{-1})	
$N\text{-}NO_3^-$	210	Fe	1150
$N\text{-}NH_4^+$	14	Mn	399
K	391	Zn	150
P	62	Cu	150
Mg	49	B	500
Ca	385	Mo	48
S	232		

NS, nutrient solution.

240 mg · L^{-1} P_2O_5, 270 mg · L^{-1} K_2O, 100 mg · L^{-3} Mg, <10% dry matter, 80–85% water capacity, 5–10% air capacity, pH (H_2O, v/v 1:1.25) = 6.0, EC = 0.45 dS · m^{-1}, dry density = 160 kg · m^{-3}. The trays were placed in the greenhouse and watered daily for 3 weeks until successful rooting. Then the plants were trimmed to obtain a uniform height and two trays containing 36 peppermint and 36 spearmint plants were placed in each basin.

The NSs were stirred frequently to ensure adequate aeration of the roots. After stirring, the pH and EC were recorded using a portable instrument (C5020, Consort, Turnhout, Belgium).

Twenty plants in each treatment were harvested after 24 days while still being at the vegetative herbaceous stage. Plant height and root length were recorded *in planta* before harvest, while plant weight was measured immediately after harvest. A total of 50 g and 10 g of fresh tissue from each treatment were used for the essential oil extraction and polyphenolic analysis, respectively. The remaining tissue was kept at −20 °C for compositional analysis.

Frozen tissue of peppermint and spearmint was homogenised in a waring blender, a mortar and pestle; and the homogenised tissue was used to determine the composition and total antioxidant capacity.

Dry matter was determined after drying 30 g of homogenised tissue at 70 °C for 72 h. The total soluble solids were determined with the use of a portable digital refractometer (PAC-1, Atago Co Ltd., Tokyo, Japan) in juice obtained after squeezing the homogenised tissue.

Nitrate content was determined following the method of Cataldo et al. (1975).

The total antioxidant capacity was determined following the method of Brand-Williams et al. (1995) using an ascorbic acid standard curve and expressed as milligram ascorbic acid equivalents (AEAC) · 100 g^{-1} in fresh weight (f.w.), while the total soluble phenol content was determined following the method of Scalbert et al. (1989), using a gallic acid curve and expressed as milligram gallic acid equivalents (GAE) · kg^{-1} in f.w., on a Thermo Spectronic Helios Alpha UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

For the extraction of pigments, 10 mL acetone was added to 0.2 g of homogenised tissue and incubated at −20 °C overnight. The following day, total chlorophyll and total carotenoids content were determined according to the method of Lichtenthaler and Wellburn (1983).

Essential oil

The essential oil content was determined on hydrodistillation of 50 g samples of fresh tissue previously cut into small pieces, using a European Pharmacopoiea apparatus (Clevenger-type) for 2.5 h, with a distillation rate of 3–3.5 mL · min^{-1}. The essential oil was dried over anhydrous sodium sulphate and was stored at 4–6 °C until analysis.

The essential oil was analysed by gas chromatography-mass spectroscopy (GC-MS) on a Shimadzu GC 17A Ver. 3 coupled with Mass Spectrometer QP-5050A (Shimadzu Europa GmbH, Germany), supported by Class 5000 software. The analysis was performed on a fused silica DB-5 capillary column, with the following conditions: injection point temperature 260 °C, ion source temperature 200 °C, GC-MS connection temperature 300 °C, Electron Ionisation 70 eV, scanning range 41–450 amu and scanning time 0.50 s. The oven temperature programmes applied were (a) 55–120 °C (3 °C · min^{-1}), 120–200 °C (4 °C · min^{-1}), 200–220 °C (6 °C · min^{-1}) and 220 °C for 5 min, and (b) 60–240 °C (3 °C · min^{-1}). The carrier gas was He, 54.8 kPa and the sample inlet ratio was 1:30.

The relative to *n*-alkanes (C8–C20) retention indices (RI) of the compounds were used for their identification, for comparing them with the respective reference substances (Adams, 2007) and for comparing the spectra with similar mass spectra of the MS libraries NIST 98 and Wiley 1995. The relative content of each compound was calculated as a percentage of the total chromatographic area.

Polyphenolic compounds

Samples of 10 g of the fresh plant tissue of each treatment was freeze-dried (Freeze-dryer Alpha 1–2 LD plus, Christ, Osterode, Germany) at −24 °C, and pulverised to fine powder; subsequently, 200 mg of freeze-dried tissue powder was extracted with 10 mL 80% methanol into 15 mL falcon tubes. The samples were mixed on an orbital shaker for 3 h at room temperature and stored overnight at 4 °C in the dark. The extracts were filtered on a MILLEX 0.22 mm PFTE membrane (Merck Millipore, Darmstadt, Germany) into a glass vial and were analysed for polyphenolics composition in an ultra-performance liquid chromatography coupled with mass spectrometer (UPLC-MS/MS), as described below.

Targeted UPLC-MS/MS analysis was performed on a Waters Acquity system (Milford, MA, USA), consisting of a binary pump, an online vacuum degasser, an autosampler and a column compartment. Separation of the phenolic compounds was achieved on a Waters Acquity HSS T3 column (1.8 mm, 100 mm × 2.1 mm), kept at 40 °C. The analysis of the phenolic compounds was performed using the method described previously by Vrhovsek et al. (2012). Mass spectrometry detection was performed on a Waters Xevo TQMS instrument equipped with an electrospray (ESI) source. Data processing was performed using the Mass Lynx Target Lynx Application Manager (Waters).

Experimental design and statistical analysis

Analysis of variance of the data was performed in SPSS v.24 (IBM, USA) using a completely randomised design with three replicates per NaCl concentration. Means were compared with the least significant differences (LSD) test at $p < 0.05$.

RESULTS

EC and pH of NS

Figure 1 illustrates the EC (A) and pH (B) in the NSs during the cultivation of plants. The EC of the two salinity treatments (10 mM and 20 mM NaCl) was significantly higher (4.2 mS · cm^{-1} and 5.0 mS · cm^{-1}, respectively) than that of the control (3.5 mS · cm^{-1}), which followed the same pattern, remaining constant for most part of the cultivation period, beginning to rise only after 18 days, and reaching the maximum levels on the day of harvest (5.8 mS · cm^{-1}, 7.0 mS · cm^{-1} and 7.5 mS · cm^{-1}, respectively, for 0 mM, 10 mM and 20 mM NaCl) (Figure 1A). The pH of the NS in all treatments was 7.1–7.2 at the beginning of the cultivation period and following the same trend it was constantly declining, reaching the lowest value of 6.6 in all solutions 1 week before harvest, when it began to rise again rapidly, climbing up to the initial values (Figure 1B).

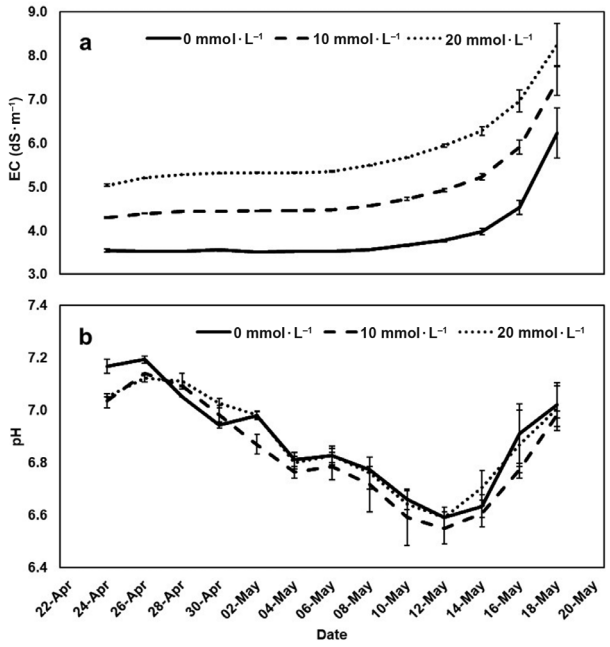

Figure 1. EC (A) and pH (B) of the NSs supplemented with 0 mmol · L^{-1}, 10 mmol · L^{-1} or 20 mmol · L^{-1} NaCl during peppermint and spearmint cultivation in floating system. Each value is a mean of three replicates and each replicate consists of one basin. EC, electrical conductivity; NSs, nutrient solutions.

Plant growth and yield

The addition of NaCl in the NS did not affect the f. w. or the dry matter in both peppermint and spearmint, but the plant height was significantly decreased by 18.5% and 11.8%, respectively, only when the plants were grown on the highest salinity levels (Table 2). Furthermore, root length was significantly reduced only in spearmint plants at the highest salinity, as well (Table 2).

Composition

Sodium chloride supplement in the NSs of peppermint plants did not affect their composition (Table 2). In spearmint plants, however, the addition of 20 mmol · L^{-1} NaCl in the NS induced significant changes in several components. In particular, the nitrate content had decreased, while the total antioxidant capacity, the total soluble phenols and the total carotenoids had increased. Moreover, the essential oil content of spearmint

Table 2. Plant growth and the compositional characteristics of peppermint and spearmint plants grown in floating system under three NaCl concentrations (0 mmol · L^{-1}, 10 mmol · L^{-1} or 20 mmol · L^{-1}).

	Peppermint				Spearmint			
	NaCl concentration				NaCl concentration			
	0 mmol · L^{-1}	10 mmol · L^{-1}	20 mmol · L^{-1}	*p*	0 mmol · L^{-1}	10 mmol · L^{-1}	20 mmol · L^{-1}	*p*
Fresh weight (g)	10.2[x]	9.35	8.05	ns[y]	8.05	7.42	6.82	ns
Plant height (cm)	22.83 a[z]	21.13 a	18.61 b	**	24.06 a	23.32 a	21.23 b	*
Root length (cm)	12.97	14.41	12.81	ns	11.48 a	11.26 a	9.55 b	*
Dry matter (%)	14.2	13.9	15.1	ns	17.2	17.8	19.8	ns
Total soluble solids (%)	9.5	8.8	9.7	ns	9.5	9.7	10.2	ns
Nitrates (mg · kg^{-1} f.w.)	828.5	855.7	766.6	ns	797.3 a	721.4 a	548.9 b	*
Total antixidant capacity (mg AEAC · 100 g^{-1} f.w.)	110.3	93.8	160.7	ns	65.0 b	78.6 b	115.7 a	**
Total soluble phenols (mg GAE · kg^{-1} f.w.)	0.467	0.417	0.663	ns	0.367 b	0.447 b	0.577 a	**
Total chlorophyll (µg · g^{-1} f.w.)	1138.4	1130.0	1133.9	ns	1173.4	1241.6	1287.7	ns
Total carotenoids (µg · g^{-1} f.w.)	200.4	196.5	201.9	ns	204.7 b	218.4 b	234.8 a	**
Essential oil (ml · 100 g^{-1} f.w.)	0.34	0.35	0.37	ns	0.19 b	0.21 a	0.22 a	*

[x] Values are means of three replicates.

[y] *, **, *** shows significant differences at 0.05, 0.01 and 0.001 probability levels; ns shows non-significant differences.

[z] Values in the same row followed by different letters differ significantly by LSD test ($p < 0.05$).

increased in both salinity levels by 10.5% and 15.8%, respectively. Neither the total soluble solids nor the total chlorophyll content were affected by the salinity treatments (Table 2).

Essential oil and polyphenolic composition

The addition of NaCl in the NS did not affect either the essential oil (Table 3) or the polyphenolic composition (Table 4) of both peppermint and spearmint fresh tissue. The only significant difference observed in the compounds of the essential oil of spearmint was of 3-octanol, which was increased under NaCl treatment, but its individual concentration is quite low in relation to the overall composition of the essential oil.

DISCUSSION

In the floating system, as water and nutrient uptake is a continuous function during plant growth and development, an NS depletion as well as a nutrient imbalance may be exhibited due to the absorption of specific ions at a higher rate than others, thus causing a modification of both EC and pH of the solution. A rapid increase in EC may be observed when low quality saline water is used, especially in arid and semi-arid areas, as non-essential ions as Na^+ and Cl^- accumulate in the NS (Urrestarazu and García, 2000). Changes in the pH of the NS are also induced, as roots release H^+ and HCO_3^- ions during nutrient uptake (Hinsinger et al., 2003). In the present study, the rapid increase of EC and the changes in the pH of the solutions cannot be attributed solely to the presence of NaCl, as the control solution followed a similar trend, and therefore may also be attributed to the NS depletion due to evapotranspiration and the change of ionic ratios (Urrestarazu and García, 2000).

Extreme levels of salinity impose a harmful effect on plant growth; the most common morphological, physiological and biochemical parameters that indicate salinity stress are stunted growth, water stress, disruption of metabolic processes and ion uptake, high respiration, synthesis of osmoregulators, disruption of photosynthesis, ion imbalance and inhibition of enzyme activity (Estaji et al., 2018). Although a decrease in plant height was observed in both peppermint and spearmint plants and in root length of spearmint, the fresh and dry biomass and the total soluble solids content were not affected by the presence of NaCl in the NS, possibly because of the low levels of salinity and the short period of cultivation. The hydroponic production of herbs in NS of medium to high salinity (20–130 mM NaCl) has shown various responses in plant growth, depending on the species or cultivar, the level of salinity, the type of salt and the period of abiotic stress. Indeed, a negative effect on plant growth was observed in herbs, such as basil, marjoram, mint *timija*, sage, lavender and mint, grown either hydroponically under salinity or in pots and irrigated with NS with increased EC due to NaCl presence (Bernstein et al., 2010; Jelali et al., 2011; Taârit et al., 2011; Tarchoune et al., 2013; Kasrati et al., 2014; Yu et al., 2015; García-Caparrós et al., 2017). The decrease in biomass and yield in plants treated with high amounts of NaCl may be attributed to stomatal limitations, which in turn restrict carbon dioxide (CO_2) diffusion into the leaf and induce further osmotic and salt-specific effects, as reported by Noecleous et al. (2017) in melon (*Cucumis melo* L.) plants grown hydroponically in recirculating NS.

Although the chlorophyll content may be used as an indicator of salt stress, it is affected differently depending on the plant sensitivity and may either decrease or remain unaffected in sensitive species and even increase in tolerant ones (Jungklang, 2003; Lee et al., 2004; Qiu and Lu, 2003). Similar to our results, the chlorophyll content in basil plants was not affected by salinity up to 130 mmol · L^{-1} NaCl, implying that it is not a reliable indicator of salinity stress for these species.

One quality trait of high importance in green leafy herbaceous plants is their nitrate content. The accumulation of nitrates has triggered the attention of the medical society, because it has been associated with severe human health risks and many countries have adopted regulations setting the maximum accepted nitrate levels in many species produced for human consumption (Santamaria, 2006). The decrease in nitrate content that was observed in spearmint plants may be attributed to the competition between nitrate and chloride ions for the same transporter in the root system (Blom-Zandstra and Lampe, 1983). A similar decrease in nitrate content has been observed in celery grown hydroponically under high saline conditions (50–300 mmol · L^{-1} NaCl) (Pardossi et al., 1999), as well as in lettuce grown in a floating hydroponic system after addition of 5,000–10,000 mmol · L^{-1} $CaCl_2$ in the NS (Borghesi et al., 2013).

Salinity stress may also have a positive or negative impact in the production of secondary metabolites in plants (Verma and Shukla, 2015; Thakur et al, 2019) and may improve their antioxidant capacity by increasing the synthesis of compounds that act as scavengers, protecting the cells against reactive oxygen species (Dat et al., 2000). Several secondary metabolites, such as carotenoids, polyphenols and essential oil components, are important antioxidants in herbs (Wojdyło et al., 2007; Riachi and De Maria, 2015). Irrespective of the NaCl concentration in the NSs, the main constituents in the essential oil of peppermint were menthone (55%), menthol (15%), menthofuran (6.5%) and isomenthone (6%) (Table 3) and in the spearmint were carvone (40%), limonene (17.5%), 1–8 cineole (16.5%) and myrcene (4.5%). Higher contents of carvone (50–57%) and lower of limonene (7–10%) were found in the aerial parts of spearmint plants that were collected from naturally growing populations (Chauhan et al., 2010; Zekri

Table 3. Essential oil composition of peppermint and spearmint plants grown in floating system under three NaCl concentrations (0 mmol · L^{-1}, 10 mmol · L^{-1} or 20 mmol · L^{-1}).

			Peppermint				Spearmint			
			NaCl concentration				NaCl concentration			
No.	compounds	RI[a]	0 mmol · L^{-1}	10 mmol · L^{-1}	20 mmol · L^{-1}	*p*	0 mmol · L^{-1}	10 mmol · L^{-1}	20 mmol · L^{-1}	*p*
1	α-thujene	929	0.03[b]	0.03	0.03	ns[c]	0.02	0.02	0.02	ns
2	α-pinene	935	0.60	0.61	0.61	ns	1.23	1.23	1.26	ns
3	camphene	950	-	-	-		0.02	0.01	0.02	ns
4	sabinene	974	0.42	0.43	0.43	ns	1.66	1.71	1.71	ns
5	β-pinene	977	0.90	0.90	0.91	ns	2.52	2.53	2.60	ns
6	1-octen-3-ol	982	0.02	0.01	0.01	ns	-	-	-	
7	myrcene	992	0.25	0.25	0.24	ns	4.65	4.46	4.47	ns
8	3-octanol	996	0.07	0.07	0.05	ns	0.72 b[d]	0.79 a	0.81 a	*
9	pseudolimonene	1004	-	-	-		0.11	0.10	0.11	ns
10	α-terpinene	1016	0.26	0.22	0.24	ns	0.04	0.01	0.03	ns
11	*o*-cymene	1025	0.03	0.05	0.03	ns	-	-	-	
12	limonene	1029	2.22	2.30	2.32	ns	17.57	17.99	17.97	ns
13	1,8-cineol	1032	4.14	4.11	4.29	ns	16.44	16.34	16.86	ns
14	*cis*-β-ocimene	1040	0.14	0.14	0.13	ns	0.61	0.59	0.62	ns
15	*trans*-β-ocimene	1051	-	-	-		0.12	0.13	0.14	ns
16	γ-terpinene	1060	0.46	0.39	0.44	ns	0.07	0.05	0.06	ns
17	cis-sabinene hydrate	1068	0.37	0.44	0.42	ns	0.01	0.03	0.02	ns
18	terpinolene	1087	0.12	0.11	0.11	ns	0.09	0.08	0.08	ns
Monoterpene hydrocarbons (%)			10.00	10.06	10.27		45.88	46.07	46.77	
19	linalool	1100	0.10	0.10	0.09	ns	0.11	0.12	0.10	ns
20	*trans*-pinocarveol	1139	-	-	-		0.15	0.18	0.16	ns
21	menthone	1158	55.44	55.98	54.51	ns	-	-	-	
22	menthofuran	1164	6.49	5.64	6.15	ns	-	-	-	
23	isomenthone	1166	6.25	6.79	6.61	ns	-	-	-	
24	neomenthol	1167	0.12	0.12	0.12	ns	-	-	-	
25	δ-terpineol	1167	-	-	-		0.45	0.47	0.47	ns
26	menthol	1176	15.16	15.24	15.30	ns	-	-	-	
27	terpinen-4-ol	1177	1.27	1.15	1.21	ns	0.13	0.11	0.14	ns
28	neoisomenthol	1182	0.07	0.07	0.07	ns	-	-	-	
29	α-terpineol	1189	0.14	0.13	0.13	ns	0.91	0.94	0.92	ns
30	dihydrocarveol	1193	-	-	-		0.11	0.09	0.08	ns
31	*trans*-dihydrocarvone	1194	-	-	-		0.12	0.13	0.12	ns
32	*trans*-carveol	1219	-	-	-		1.08	0.99	0.95	ns
33	pulegone	1238	1.76	1.68	1.73	ns	-	-	-	
34	carvone	1247	-	-	-		40.64	40.54	39.49	ns
35	piperitone	1253	0.49	0.50	0.50	ns	-	-	-	
36	dihydroedulan II	1284	-	-	-		0.13	0.14	0.18	ns
37	menthyl acetate	1294	0.42	0.58	0.65	ns	-	-	-	
Oxygenated monoterpenes (%)			87.71	87.97	87.08		43.83	43.71	42.61	
38	α-copaene	1376	-	-	-		0.02	0.03	0.03	ns
39	β-bourbonene	1383	-	-	-		0.49	0.54	0.58	ns
40	β-elemene	1391	-	-	-		0.18	0.18	0.19	ns
41	β-caryophyllene	1415	0.60	0.54	0.74	ns	3.82	2.69	3.89	ns
42	α-humulene	1451	-	-	-		0.17	0.17	0.18	ns
43	*cis*-β-farnesene	1461	0.11	0.10	0.13	ns	0.11	0.11	0.11	ns
44	germacrene-D	1479	1.01	0.84	1.17	ns	4.35	4.03	4.22	ns
45	bicyclogermacrene	1492	0.09	0.07	0.10	ns	0.37	0.35	0.38	ns
46	δ-cadinene	1525	-	-	-		0.09	0.09	0.10	ns
Sesquiterpene hydrocarbons (%)			1.82	1.54	2.14		9.61	8.20	9.67	
47	caryophyllene oxide	1584	-	-	-		0.04	0.02	0.04	ns
48	viridiflorol	1591	0.26	0.25	0.26	ns	-	-	-	

(*Continued*)

Table 3. Continued

No.	compounds	RI[a]	Peppermint NaCl concentration 0 mmol · L^{-1}	Peppermint NaCl concentration 10 mmol · L^{-1}	Peppermint NaCl concentration 20 mmol · L^{-1}	*p*	Spearmint NaCl concentration 0 mmol · L^{-1}	Spearmint NaCl concentration 10 mmol · L^{-1}	Spearmint NaCl concentration 20 mmol · L^{-1}	*p*
Oxygenated sesquiterpenes (%)			0.26	0.25	0.26		0.04	0.02	0.04	
49	13-epi-manool oxide	2012	-	-	-		0.12	0.17	0.18	ns
Oxygenated diterpenes (%)			0.00	0.00	0.00		0.12	0.17	0.18	
Total (%)			99.79	99.82	99.75		99.48	98.18	99.27	

[a] RI: retention indices relative to n-alkanes on a DB-5 column.

[b] Values are means of three replicates.

[c] *, **, *** shows significant differences at 0.05, 0.01 and 0.001 probability levels; ns shows non-significant differences.

[d] Values in the same row followed by different letters differ significantly by LSD test ($p < 0.05$).

Table 4. Polyphenolic composition of peppermint and spearmint plants grown in floating system under three NaCl concentrations (0 mmol · L^{-1}, 10 mmol · L^{-1} or 20 mmol · L^{-1}).

No.	compounds (mg · 100 g^{-1} d.w.)	Peppermint NaCl concentration 0 mmol · L^{-1}	Peppermint NaCl concentration 10 mmol · L^{-1}	Peppermint NaCl concentration 20 mmol · L^{-1}	*p*	Spearmint NaCl concentration 0 mmol · L^{-1}	Spearmint NaCl concentration 10 mmol · L^{-1}	Spearmint NaCl concentration 20 mmol · L^{-1}	*p*
1	Vanillic acid	0.34[a]	0.34	0.27	ns[b]	0.84	0.78	0.71	ns
2	2,6-dihydroxybenzoic acid	0.68	0.39	0.51	ns	0.77	0.61	0.77	ns
3	Syringaldehyde	0.03	0.02	0.05	ns	0.06	0.03	0.04	ns
4	Daphetin	0.13	0.14	0.12	ns	0.13	0.15	0.13	ns
5	Caffeic acid	11.79	9.88	12.45	ns	13.23	14.18	16.72	ns
6	Ferulic acid	0.23	0.32	0.24	ns	0.29	0.26	0.24	ns
7	Caftaric acid	19.35	17.40	23.29	ns	13.73	18.10	15.76	ns
8	Neochlorogenic acid	5.24	4.72	5.45	ns	3.06	3.64	3.62	ns
9	Chlorogenic acid	4.62	4.89	5.97	ns	4.47	6.18	5.43	ns
10	Rosmarinic acid	287.81	257.40	373.28	ns	417.62	489.75	491.95	ns
11	Sinapyl alcohol	0.68	0.59	0.64	ns	0.56	0.37	0.58	ns
12	Fertaric acid	1.80	1.72	1.85	ns	2.60	3.34	2.74	ns
13	*t*-coutaric	0.76	1.17	1.29	ns	1.14	1.41	1.20	ns
14	Apigenin	1.57	1.86	1.83	ns	0.55	0.62	0.95	ns
15	Luteolin	7.50	7.14	8.38	ns	1.85	2.30	2.62	ns
16	Luteolin-7-*O*-glucoside	0.88	0.98	1.02	ns	1.18	1.57	1.54	ns
17	Hesperidin	128.98	124.94	149.51	ns	88.72	78.69	88.95	ns
18	Apigenin-7-glucoside	0.21	0.28	0.31	ns	0.43	0.65	0.64	ns
19	Naringenin	1.90	2.05	2.76	ns	1.08	1.27	1.18	ns
20	Kaempferol-3-glucoside	0.02	0.01	0.01	ns	0.02	0.05	0.03	ns
21	Arbutin	0.07	0.08	0.08	ns	0.13	0.11	0.08	ns
22	Syringic acid	0.76	0.68	0.61	ns	0.47	0.62	0.38	ns
23	Cryptochlorogenic acid	16.22	16.78	20.73	ns	15.40	21.39	19.05	ns
24	Quercetin-3-glucoside	0.23	0.15	0.26	ns	0.34	0.32	0.35	ns
25	Quercetin-4-*O*-glucoside	40.03	38.67	47.29	ns	26.43	22.22	26.76	ns
26	Rutin	1.09	1.26	1.52	ns	2.47	1.82	1.73	ns

[a] Values are means of three replicates.

[b] *, **, *** shows significant differences at 0.05, 0.01 and 0.001 probability levels; ns shows non significant-differences.

et al., 2019; Farahbakhsh et al., 2021), which may be explained by different genotypes, location or agronomic conditions, such as harvesting time, plant age, crop density, as well as growing substrate (soil against soilless NS). Rosmarinic acid and hesperidine were the main phenolic components in both peppermint and spearmint plants, which is in line with the findings of a more recent study in Iran (Farahbakhsh et al., 2021), which, too, observed significant antioxidant activity.

In our study, an increase in the above antioxidant components was observed only in spearmint grown in NS containing 20 mmol · L^{-1} NaCl. Similar results have been observed in the carotenoid content of basil plants grown under different levels of saline conditions (Bernstein et al., 2010), as well as in the total phenolic content of marjoram and sage and the total antioxidant capacity of the latter (Jelali et al., 2011; Taârit et al., 2012), where the content of these components increased relatively while increasing the levels of salinity. Phenolics content and

antioxidant capacity in lettuce plants grown in a floating hydroponic system and harvested 5 weeks after sowing were favoured by the moderate salt conditions induced after $CaCl_2$ supplement in the NS (Borghesi et al., 2013).

High salinity may also influence individual polyphenolics' composition, as observed in rosemary plants grown under NSs where various salts had been added, but in the NaCl treatment no differences were found in phenolic diterpene concentrations (Tounekti et al., 2011), which is similar to the findings of the present study. Many researchers have reported contradictory results regarding variations in the essential oil content and its constituents in herbs grown under saline conditions. In less resistant species, like the Canadian mint and mint *timija*, a decrease in the essential oil content was observed when grown in high salinity of >50 mmol · L^{-1} NaCl (Kasrati et al., 2014; Yu et al., 2015). However, in more resistant species, such as marjoram, sage and basil, mild salinity stress may induce the production of essential oil until a maximum limit of salinity level and beyond that a decrease was observed (Bernstein et al., 2010; Jelali et al., 2011; Taârit et al., 2011). The type of salt also constitutes an important factor, as the essential oil content of basil plants decreased while grown in NS containing 25 mmol · L^{-1} Na_2SO_4 and increased while grown in 50 mmol · L^{-1} NaCl (Tarchoune et al., 2013). Changes in the essential oil content were often accompanied by a simultaneous modification in the relative concentration of the essential oil constituents, resulting in a significant effect on the oil's compositional profile.

CONCLUSIONS

In conclusion, peppermint and spearmint are suitable for a sustainable environmentally friendly soilless production in the floating system under mild salinity conditions (10 mmol · L^{-1} to 20 mmol · L^{-1} NaCl), without any adverse effects on the fresh biomass yield, the nutritional characteristics or the essential oil content and composition. Indeed, spearmint benefits from a slight salinity stress increase in the NS, as the nitrate content decreased and the secondary metabolites increased. Both species may be grown in greenhouse production units that do not have access to high quality water. The essential oil and the phenolic composition of both peppermint and spearmint were not affected, indicating that these two species may profitably be grown even under 20 mmol · L^{-1} NaCl without suffering from oxidative stress or from risking any market value loss.

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AUTHOR CONTRIBUTIONS

D-C.A., P.T. and A.S.S.: conceptualisation. P.T., D-C.A., D.S.K. and E.S.: methodology. P.T., D-C.A. and E.S.: software. A.S.S., P.C. and S.M.: formal analysis. D.S.K. and D-C.A.: investigation. P.T., D-C.A. and E.S.: data curation. P.T, D.S.K, D-C.A. and E.S.: writing and original draft preparation. S.M, P.C. and A.S.S.: writing, review and editing. P.T. and A.S.S.: supervision.

CONFLICT OF INTEREST

All authors declare that no conflicts of interest exist.

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