Industrial Crops and Products 92 (2016) 50–56

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Enhancing mint and basil oil composition and antibacterial activity using seaweed extracts

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ARTICLE INFO

Article history:
Received 7 March 2016
Received in revised form 29 July 2016
Accepted 29 July 2016
Available online 6 August 2016

Keywords:
Mint
Basil
Seaweeds extracts
Essential oil

ABSTRACT

The increasing cost of synthetic fertilizers and conventional agrochemicals calls for an urgent search for next generation of environmental-friendly competitive fertilizers and growth stimulants that enhance the essential oil content and composition of traditional global medicinal plants such as mint (*Mentha* × *piperita* L."chocolate") and sweet basil (*Ocimum basilicum* L. "purple ruffle"). The study aims to evaluate the morphological and physiological effects of seaweed extracts (*Ascophyllum nodosum*) doses and application methods on mint and basil plants essential oil composition and its respective antibacterial activities. The plants were subjected to two doses of foliar/drench weekly applications of *A. nodosum* extracts at 5 and 7 mL L^{-1} for 12 weeks. *A. nodosum* extracts drench and foliar treatments increased leaf number and area, dry weights, and plant height of both plants. In mint and basil plants, there were increases in the essential oil content and enhanced composition following *A. nodosum* treatments. In mint plants, the drench application of 7 mL L^{-1} SWE had the highest oil contents of L-menthone (32.4%) and L-menthol (32.6%) while basil treated plants showed the highest composition of chavicol methyl ether (38.7%), linalool (29.1%), and cineol (9.1%). Additionally decreasing of potentially toxic pulegone and methofuran in mint oil was noticed. *A. nodosum* treated plants showed higher antibacterial potential than control. In mint and basil plants, the highest antibacterial activity found was in the essential oil of mint plants drenched with 7 mL L^{-1} SWE and the antibacterial activities of mint oils were higher than basil. The biostimulant effect of *A. nodosum* extract treatments was attributed to the macro- and micro-elements composition as well as the carbohydrate contents.

1. Introduction

The family Lamiaceae contains some of the most important and traditionally known medicinal plants used as food or medicine such as mint (*Mentha* sp.) and sweet basil (*Ocimum basilicum* L.). The Mediterranean region represents "hot spots" of production and consumption of both taxa (FAOSTAT, 2016). Mint plants are used either raw as soft drink and for flavoring and culinary purposes or processed for generating oils used in cosmetic and pharmaceutical industries (Grigoleit and Grigoleit, 2005; Zheljazkov et al., 2010; Elansary and Mahmoud, 2015a; Elansary et al., 2015). Mint oil, globally known as peppermint oil, which refers to *Mentha piperita* L., is one of the mostly used species for oil production (Skalicka-Woźniak and Walasek, 2014). Peppermint oil has unique antibacterial, antifungal, antioxidant properties (Mimica-Dukić et al., 2003; Yadegarinia et al., 2006). Basil plants are used either fresh in garnishing foods or after being processed for essential oil production. Also, basil oil is commonly used for traditional herbal remedies and has antibacterial, antifungal and antioxidant activities (Yavari et al., 2011; Azizkhani and Parsaeimehr, 2015; Elansary and Mahmoud, 2015b).

Modifying and enhancing the essential oil content and composition of mint and basil, as well as several industrial crops, has increasingly been the focus of several recent studies world-

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http://dx.doi.org/10.1016/j.indcrop.2016.07.048
0926-6690/

wide. These studies employed several techniques such as genetic engineering (Lange et al., 2011), bacteria and mycorrhiza (Singh et al., 2013; Weisany et al., 2015), mineral fertilizers and stress (Jeshni et al., 2015) and irrigation regimes (Alinian et al., 2016). Although the application of seaweed extracts (SWE) is one of the common methods to improve plant growth characteristics, it has never been applied on mint and basil as essential oil crops. Seaweeds are marine algae that naturally grow at the coastal regions of the world with a global economic value of around 6 billion USD (FAOSTAT, 2014). From the agricultural industry perspective, they are considered as alternative organic fertilizers to conventional agrochemicals, new generation of competitive fertilizers and growth stimulants (Sharma et al., 2014; Elansary et al., 2016). Some studies indicated that SWE may act as partial substitution for fertilizers (Dhargalkar and Pereira, 2005; Hong et al., 2007; Zodape et al., 2010) because they may contain minor and major elements. Saccharides contained in SWE may act as elicitors of plant defensive mechanisms (Anastyuk et al., 2009; Stengel et al., 2011; Vera et al., 2012). One of the most important and globally known SWE is the extracts obtained from the brown algae *Ascophyllum nodosum* (L.) Le Jolis (Fucaceae). Several studies indicated that foliar and drench applications of *A. nodosum* SWE enhanced the growth of field crops (Steveni et al., 1992; Blunden et al., 1996), fruit crops (Chouliaras et al., 2009; Spinelli et al., 2009; Little and Spann, 2010; Khan et al., 2012) and vegetable crops (Jayaraj et al., 2008; Neily et al., 2010). These studies reported also an improved vegetative growth, chlorophyll content, fruit yield, sugar content and resistance against leaf and soil borne pathogens. However, studies on medicinal aromatic crops are still lacking.

The purpose of this study is to evaluate how the application of SWE at different doses affects the morphological and physiological characteristics of mint and basil plants, following two different application methods. Specifically we assess the change in essential oil content and composition, as well as antibacterial activities of oils of both plants following the application of SWE. Economic morphological parameters such as leaf number and area, plant dry weight, root dry weight, and plant height were selected as well as essential oil content and composition. Additionally the antibacterial activities of the essential oils of treated and non-treated plants against wide-spectrum bacterial were illustrated. The mineral and sugar composition of SWE were also evaluated and discussed.

2. Material and methods

2.1. Plant materials and growing conditions

Mentha × *piperita* L. the cultivar "chocolate" and *Ocimum basilicum* L. the cultivar "purple ruffle" plants are known cultivars worldwide were obtained from local commercial nurseries. *Mentha* × *piperita* is a hybrid between *Mentha aquatica* and *Mentha spicata*, also purple ruffle is a known commercial cultivar of basil. The plants were identified by Dr. Elansary and vouchered at the Biodiversity Institute of Ontario (No. Hosam994-Hosam1143). The plants were transplanted into 2.1 L pots and each pot contained one plant. The growing media was black peat, coconut fiber, and perlite (1:1:1) fertilized with Osmocote Plus (14:13:13 N, P, K +microelements) (2 g L^{-1} media). The experiment was performed in controlled greenhouse conditions located in Guelph, Ontario, Canada (43° 30′ 18.24″ N 80° 22′ 15.86″ W). The plants were watered by drip irrigation for the full pot capacity during the experiment that continued for 3 months. The temperatures ranged between 23.1 and 30 °C, the mean relative humidity ranged between 56 and 67%, and the photosynthetically active radiation was 1000 μmol m^{-2} s^{-1} at 10:00 a.m.

2.2. Treatments

Each cultivar was subjected to two doses of foliar weekly applications of *Ascophyllum nodosum* extracts (Stella Maris ™, Acadian Seaplants, Canada, 2015, Patch No. 2475) at 5 and 7 mL L^{-1} until run off. Also, a soil drench at 5 and 7 mL L^{-1} of the seaweed extracts was used in other treatments. Untreated plants were considered as control. Plants were grouped into three repetitions (n = 3), with 5 plants per treatment, making it a total of 75 plants per cultivar distributed in three blocks.

2.3. Measurements

Data were collected at the end of the experiment in June 2015 after 12 weeks of SWE treatments. Plant heights were recorded, then the whole plants were harvested and the substrate was washed delicately from the roots. Leaf numbers of all plants were calculated and the area was measured using Delta-T Devices Ltd., Cambridge, UK. Total dry weight and root dry weight were determined immediately after morphological parameters calculation, by oven-drying at 35 °C to reach constant weight.

2.4. Isolation of essential oil and gas chromatography/mass spectrometry (GC/MS)

Dried leaves of each plant (4.1–12.5 g) were immediately ground and hydro-distilled in Clevenger-type apparatus for 2 h, then essential oils were dried over anhydrous sodium sulfate, filtered and stored in sealed vials at 4 °C. The analyses of essential oils were performed using Thermo Scientific Gas Chromatograph (Trace GC Ultra) coupled with Thermo Scientific Mass Spectrometer (ISQ) instrument. TG-1MS narrow bore column (30 m × 0.32 mm ID, 0.25 μm film thickness) was used for the separation and the helium was used as carrier gas. Oven temperature was programmed to increase from 45 °C to 165 °C at 4 °C min^{-1} with holding time of 2 min at 165 °C, then 15 °C min^{-1} to 280 °C, reaching a final holding time of 15 min. Each sample (2 μL) was injected at 250 °C on splitless mode flow (1 mL min^{-1}), and 3 min splitless time then 10 mL min^{-1} split flow. The GC-FID analysis was performed on the same column and temperature program. The identification of compounds was based on the retention time and retention indices related to homologous series of *n*-alkanes (C_{10}–C_{36}) analyzed on the same conditions and computer matching with the NIST mass spectral search program ver. 2.0 and WILEY libraries in additional to references from literature (Juliani and Simon, 2002; Lee et al., 2005; Adams 2007).

2.5. Antibacterial activities

The essential oils of treated and non-treated plants were examined for their antibacterial activities against multiple Gram-positive and Gram-negative bacterial strains. *Bacillus cereus* (ATCC 14579), *Listeria monocytogenes* (clinical isolate), *Staphylococcus aureus* [ATCC (American type culture collection) 6538] and *Micrococcus flavus* (ATCC 10240) were used as Gram-positive bacteria. In addition, *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 35210) were used as Gram-negative bacteria. The microdilution method using a 96-well microtitre plates was used to determine the minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations (Espinel-Ingroff, 2001). The bacterial viable count was adjusted to 1.0×10^5 CFU mL^{-1} using sterile saline and stored at 4 °C. Screening for contamination was performed by culturing on solid medium.Serial dilutions of hydro-distilled essential oils in 100 μL Triptic Soy broth (TSB) containing bacteria inoculum (1.0×10^4 CFU per well) were used to determine the MICs and MBCs of each oil. After incubation of the microplates at

37 °C for 24 h in a rotary shaker, the lowest concentration that completely inhibited bacterial growth (at the binocular microscope) was defined as the MIC while the lowest concentration indicating killing of 99.5% of the original inoculum was defined as the MBC. The MBC was determined by serial sub-cultivations of 2 μL into microtitre plates containing 100 μL of TSB for each well and incubated for 24 h. To determine the optical density a microplate manager was used at 655 nm. All experiments were in triplicates and repeated three times. Positive controls (streptomycin (SPM) and ampicillin (AMP), 0.01–10 mg mL^{-1}) and negative control (5% DMSO) were used.

2.6. Seaweed extract chemical composition

Minerals were determined using AOAC method No. 968.08 using Inductively Coupled Plasma Spectroscopic Analysis (ICPSA) in Optima 4300DV (Perkin-Elmer, USA). Determination of heavy metals followed the AOAC method No. 6020A by Atomic Absorption – Hydride Generation. Total nitrogen (N) was quantified using AOAC method No. 990.03 using LECO FP-528 analyzer. Available phosphorous (P_2O_5) was determined using ammonium citrate extraction in AOAC method No. 960.08 by ICP-OES. Soluble potassium (K_2O) was determined using ammonium oxalate extraction in AOAC method No. 960.08 by ICP-OES. To determine the sugar composition, the samples were hydrolyzed using freshly prepared 1 M methanolic-HCl for 16 h at 80 °C. The released sugars were derivatized with Tri-Sil and the samples were run on GC-FID (Agilent 6890N) equipped with Supelco 2560 capillary column (100 m × 0.25 mm, 0.2 μm thickness). The detector and the split/splitless injection port were maintained at 250 °C and the oven was maintained at 180 °C. The hydrogen was used as carrier, eluted at 1.0 mL min^{-1} with a split ratio of 1:100, and the injection volume was 1 μL. The total and individual percentage of identified carbohydrates was calculated. Experiments were repeated twice in triplicates.

2.7. Statistical analyses

The data was subjected to the Least significant differences (LSD) one-way analysis of variance (ANOVA) implemented in SPSS (PASW Ver. 21) at a level of significant of $P \leq 0.05$. The results were expressed as means ± standard deviation (SD).

3. Results

3.1. Morphological responses of basil and mint to SWE treatments

There were significant effects of SWE drench and foliar treatments on leaf number and area, dry weights, and plant height of both plants (Table 1). In mint plants the application of SWE increased all morphological parameters compared to control plants. The drench application of SWE with 7 mL L^{-1} showed the highest values of leaf numbers/plant (103 leaf/plant), leaf area (177 cm^2/plant), leaves and stems dry weight (9.1 g), root dry weight (2.3 g), and plant height (22.7 cm) compared to all treatments. In basil plants, an increase in leaf number and area, dry weights and plant height was also increased compared to control plants, however not such big differences were noticed as in case of mint.

The drench application of SWE significantly enhanced all morphological parameters compared to the foliar application. In addition, both 5 and 7 mL L^{-1} drench SWE doses did not show significant differences in most morphological parameters.

3.2. Essential oil content and composition

The drench and foliar applications of SWE had significant effects on the essential oil percentage in both mint and basil (Table 1) and the highest ratio of essential oils were found in plants treated with 5 and 7 mL L^{-1} SWE as drench application compared to the foliar applications.

Main oil constitutes in control mint plant were identified as L-menthone (29.2%), L-menthol (29.1%), menthofuran (9.5%), caryophyllene (6.4%), and germacrene-D (6.4%) (Table 2). There were changes in the main constitutes of the essential oil following SWE treatments. The method and dose of application significantly affected main oil constitutes of mint plants. Drench application of SWE achieved higher values of main oil constitutes such as L-menthone and L-menthol and reduced other compounds such as the pulegone and the menthofuran, compared to foliar applications. Moreover, the drench application of 7 mL L^{-1} SWE resulted in the highest oil contents of L-menthone (32.4%) and L-menthol (32.6%) compared to other treatments. For instance, L-menthone increased from 29.2% in the control plants to 32.4% in the drench application of 7 mL L^{-1} SWE which is considered as significant increase. In basil control plants, chavicol methyl ether (38.7%), linalool (29.1%), cineol (9.1%), α-bergamotene (5%), and α-cadinol (2.3%) were identified as main oil compounds (Table 3). Significant changes in oil main constitutes such as increases in chavicol methyl ether and linalool ratios and reductions in other contents such as α-bergamotene were noticed in SWE treated plants. The drench applications of 7 mL L^{-1} SWE showed significantly the highest values of chavicol methyl ether (41.9%), linalool (31.7%), and cineol (9.8%). For instance, chavicol methyl ether increased from 38.7% in the control plants to 41.9% in the drench application of 7 mL L^{-1} SWE which is considered as significant increase. In addition, the drench application of 5 mL L^{-1} SWE showed comparable effects to the foliar applications of SWE.

3.3. Antibacterial activities of essential oil

The essential oils of mint and basil distilled from control and treated plants were screened for their antibacterial activities against number of bacteria (Table 4). All essential oils showed variable antibacterial activities against bacteria; also SWE treated plants showed higher antibacterial potential than control. In mint plants, the MIC of the essential oils ranged from 0.004 to 0.13 mg mL^{-1}, whereas the MBC ranged from 0.010 to 0.27 mg mL^{-1}. The essential oil of mint plants treated with drenched 7 mL L^{-1} SWE exhibited the highest antibacterial activity with MIC and MBC of 0.004–0.09 mg mL^{-1} and 0.010–0.19 mg mL^{-1}, respectively. The foliar application showed lower antibacterial activities compared to the drench application. In basil plants, the MIC of the essential oils ranged from 0.09 to 0.21 mg mL^{-1} whereas the MBC ranged from 0.18 to 0.44 mg mL^{-1}. The basil essential oil treated with drenched 7 mL L^{-1} SWE followed by both the drench 5 mL L^{-1} SWE and foliar 7 mL L^{-1} SWE had the highest antibacterial activity. The drench SWE treatments showed higher antibacterial potential in essential oils than foliar sprays. It was noted that the antibacterial activities of mint oils were higher than basil and slightly higher than antibiotics used as reference (Table 4).

3.4. Chemical composition of SWE

The chemical composition of dried SWE showed the presence of macroelements such as nitrogen (N, 0.4%), phosphorus (P_2O_5, 0.17%) and potassium (K_2O, 0.6%) as well as microelements such as iron, copper, zinc and boron (Table 5). In addition, there were 18.9%

Table 1
The effects of SWE treatments of 5 and 7 mL L^{-1} as a soil drench (DRE) or foliar spray (FOL) on mint and basil plants leaf numbers (leaf/plant), leaf area (cm^2/plant), leaves and stems dry weight (g/plant), root dry weight (g/plant), plant height (cm) and essential oil percentage (% of fresh weight).

		leaf numbers	leaf area	leaves and stems dry weight	root dry weight	plant height	essential oil percentage
Mentha × *piperita*	Cont.	81.2 ± 0.9d*	141 ± 3.2d	7 ± 0.4c	1.7 ± 0.2c	17 ± 0.6c	0.94 ± 0.01b
	DRE (5 mL)	95.3 ± 1b	166 ± 2.6b	8.4 ± 0.3b	2.1 ± 0.1ab	18.8 ± 0.6bc	1.05 ± 0.01a
	DRE (7 mL)	103 ± 2a	177 ± 3.3a	9.1 ± 0.2a	2.3 ± 0.1a	22.7 ± 0.5a	1.12 ± 0.02a
	FOL (5 mL)	92.1 ± 1.7c	163 ± 2.5c	8.2 ± 0.3b	2 ± 0.2b	18.8 ± 0.4bc	1.0 ± 0.01ab
	FOL (7 mL)	95.2 ± 1.6b	164 ± 2.7cb	8.3 ± 0.1b	2.1 ± 0.1ab	19.6 ± 0.5b	1.03 ± 0.01a
Ocimum basilicum	Cont.	81.4 ± 0.8c	835 ± 8.1c	14.1 ± 0.5b	2.7 ± 0.1b	46.6 ± 0.9b	0.3 ± 0.01c
	DRE (5 mL)	90.8 ± 0.8a	881± 8.5b	15.1 ± 0.5a	3 ± 0.1a	48.3 ± 0.6a	0.35 ± 0.01a
	DRE (7 mL)	91.3 ± 0.7a	911 ± 7.2a	15.2 ± 0.4a	3.1 ± 0.1a	49.2 ± 0.7a	0.36 ± 0.01a
	FOL (5 mL)	86.8 ± 0.6b	863 ± 9.2b	14.4 ± 0.2ab	2.9 ± 0.1ab	46.4 ± 0.8b	0.32 ± 0.01b
	FOL (7 mL)	87.7 ± 0.7b	869 ± 7.9b	14.5 ± 0.6ab	2.8 ± 0.2b	47.5 ± 0.5ab	0.33 ± 0.01b

* Means followed by different letter within a column indicate significant differences between treatments based on LSD test ($P \leq 0.05$).

Table 2
Chemical constituents of the essential oils of *Mentha* × *piperita* chocolate as a response to SWE treatments of 5 and 7 mL L^{-1} as a soil drench (DRE) or foliar spray (FOL).

RI	LRI	Compounds	Control	DRE(5 mL)	DRE (7 mL)	FOL (5 mL)	FOL (7 mL)
1040	1040	α- Terpineol	0.5 ± 0.1b*	0.5 ± 0.1b	0.6 ± 0.0a	0.5 ± 0.1b	0.5 ± 0.1b
1051	1050	*trans*-Sabinene hydrate	1.4 ± 0.2	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.0	1.3 ± 0.1
1153	1153	L-Menthone	29.2 ± 0.2d	30.3 ± 0.1c	32.4 ± 0.1a	30.2 ± 0.2c	31.1 ± 0.1b
1163	1163	Menthofuran	9.5 ± 0.2a	9.3 ± 0.1b	9.1 ± 0.3c	9.2 ± 0.1b	9.1 ± 0.1c
1172	1171	L-Menthol	29.1 ± 0.3d	30.84 ± 0.2c	32.6 ± 0.2a	30.1 ± 0.3c	31.2 ± 0.2b
1237	1237	Pulegone	5.1 ± 0.1a	4.3 ± 0.1bc	4.4 ± 0.1b	4.2 ± 0.1c	4.1 ± 0.1c
1253	1253	Piperitone	1.3 ± 0.2	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.2	1.1 ± 0.0
1295	1295	Menthyl acetate	3 ± 0.1	2.7 ± 0.0	2.3 ± 0.1	2.5 ± 0.1	2.6 ± 0.1
1425	1425	Caryophyllene	6.4 ± 0.1a	4.2 ± 0.1b	4.1 ± 0.1b	4.2 ± 0.1b	4.2 ± 0.1b
1457	1457	*trans*-β-Farnesene	1.2 ± 0.2	0.9 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
1500	1500	Germacrene-D	6.4 ± 0.1a	4.8 ± 0.1bc	4.7 ± 0.1c	4.7 ± 0.1c	4.9 ± 0.1b
1692	1692	Veridiflorol	2.1 ± 0.3	1.8 ± 0.1	1.7 ± 0.2	1.9 ± 0.1	1.8 ± 0.2

Notes: RI: Retention index, LRI: retention index from literature.
*Means followed by different letter within a row indicate significant differences between treatments based on LSD test ($P \leq 0.05$).

Table 3
Chemical constituents of the essential oils of *Ocimum basilicum* purple ruffle as a response to SWE treatments of 5 and 7 mL L^{-1} as a soil drench (DRE) or foliar spray (FOL).

RI	LRI	compounds	Control	DRE (5 mL)	DRE (7 mL)	FOL (5 mL)	FOL (7 mL)
946	945	α-Pinene	1.2 ± 0.2	1.2 ± 0.1	1.3 ± 0.2	1.2 ± 0.1	1.2 ± 0.1
985	984	Myrcene	2.4 ± 0.2	1.7 ± 0.3	1.7 ± 0.1	1.8 ± 0.2	1.7 ± 0.1
1040	1040	Cineole	9.1 ± 0.2c*	9.5 ± 0.1b	9.8 ± 0.3a	9.4 ± 0.2b	9.8 ± 0.3a
1043	1042	Ocimene	1.2 ± 0.3	1 ± 0.1	0.9 ± 0.2	1.1 ± 0.3	0.9 ± 0.2
1092	1091	Terpinolen	0.4 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
1117	1117	Linalool	29.1 ± 0.5d	29.5 ± 0.3c	31.7 ± 0.3a	29.1 ± 0.5d	29.9 ± 0.2b
1192	1192	Terpinen-4-ol	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
1198	1199	Terpineol	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
1200	1201	Chavicol methyl ether	38.7 ± 0.4c	39.8 ± 0.3b	41.9 ± 0.5a	39.5 ± 0.5b	40.1 ± 0.3b
1379	1380	β-Elemene	2 ± 0.1a	1.6 ± 0.3b	1.1 ± 0.2c	1.6 ± 0.2b	1.5 ± 0.1b
1434	1434	α-Bergamotene	5 ± 0.3a	4.5 ± 0.2b	4.2 ± 0.3c	4.7 ± 0.2ab	4.3 ± 0.1bc
1436	1436	α-Caryophyllene	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
1438	1438	Germacrene D	1.2 ± 0.2	0.9 ± 0.1	0.9 ± 0.2	1 ± 0.2	0.9 ± 0.1
1443	1443	Caryophyllene	1.2 ± 0.3	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.0	0.8 ± 0.1
1490	1490	δ-Guaiene	1 ± 0.2	0.9 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.2
1492	1491	γ-Cadinene	1 ± 0.2	0.4 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
1624	1623	α-Cadinol	2.3 ± 0.3	2.3 ± 0.2	1.9 ± 0.1	2.0 ± 0.2	1.9 ± 0.3

*Means followed by different letter within a row indicate significant differences between treatments based on LSD test ($P \leq 0.05$).
Notes: RI: Retention index, LRI: retention index from literature.

of the dried SWE, composed of carbohydrates including mannitol (29.8%), fucose (20.3%), xylose (13.6%) and glucose (13%) (Table 6).

4. Discussion

The increases in leaf number and leaf area, dry weight and plant height concord with growth-stimulating effects of SWE reported in several studies on other horticultural crops (Kumari et al., 2011; Alam et al., 2013; Mattner et al., 2013; Hernández-Herrera et al., 2014). The essential oil composition of control plants matched those described in mint plants (see e.g. Elansary and Ashmawy, 2013) as well in basil plants (e.g. Elansary and Mahmoud, 2015b). There were increases in the essential oil content in SWE-treated plants concomitantly with increases in main constitutes such as the L-menthone and the L-menthol in mint and the linalool and chavicol methyl ether in basil. It is worth to highlight the decreasing amounts of pulegone and methofuran in mint oil after SWE treatment. Pulegone – a monoterpene ketone, as well as its metabolites menthofuran and *p*-cresol, are considered as potentially toxic and according to the regulation (EC) No 1334/2008 of the European Parliament and of the Council (EC, 2008) pulegone and menthofuran cannot be used as flavoring substances in the EU. Thus, one of the main goals of peppermint oil producers should be finding the possibility of providing peppermint oil devoid of poten-

Table 4
The antibacterial activities of the essential oils of mint and basil plants treated with SWE at 5 and 7 mL L^{-1} as a soil drench (DRE) or foliar spray (FOL). Minimum inhibitory (MIC) and bactericidal concentration (MBC) of the essential oils (mg/ml).

		L. monocytogenes	*S. aureus*	*B. cereus*	*M. flavus*	*P. aeruginosa*	*E. coli*
		MIC	MIC	MIC	MIC	MIC	MIC
		MBC	MBC	MBC	MBC	MBC	MBC
Mint	Cont.	0.13 ± 0.01	0.008 ± 0.002	0.09 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.008 ± 0.001
		0.27 ± 0.03	0.016 ± 0.001	0.17 ± 0.02	0.24 ± 0.03	0.22 ± 0.02	0.017 ± 0.003
	DRE (5 ml)	0.11 ± 0.01	0.007 ± 0.001	0.07 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.006 ± 0.001
		0.22 ± 0.03	0.015 ± 0.002	0.15 ± 0.03	0.23 ± 0.03	0.20 ± 0.03	0.013 ± 0.003
	DRE (7 ml)	0.09 ± 0.01	0.004 ± 0.001	0.05 ± 0.01	0.09 ± 0.02	0.08 ± 0.01	0.005 ± 0.001
		0.19 ± 0.01	0.010 ± 0.001	0.11 ± 0.01	0.19 ± 0.01	0.16 ± 0.01	0.011 ± 0.002
	FOL (5 ml)	0.12 ± 0.01	0.007 ± 0.001	0.08 ± 0.01	0.12 ± 0.02	0.10 ± 0.01	0.007 ± 0.001
		0.26 ± 0.03	0.014 ± 0.002	0.15 ± 0.03	0.24 ± 0.01	0.20 ± 0.03	0.014 ± 0.003
	FOL (7 ml)	0.11 ± 0.00	0.006 ± 0.002	0.07 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.006 ± 0.001
		0.21 ± 0.01	0.012 ± 0.001	0.14 ± 0.02	0.22 ± 0.03	0.19 ± 0.02	0.012 ± 0.003
Basil	Cont.	0.14 ± 0.01	0.21 ± 0.01	0.15 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.16± 0.01
		0.31 ± 0.01	0.43 ± 0.03	0.33 ± 0.03	0.23 ± 0.00	0.19 ± 0.01	0.30 ± 0.02
	DRE (5 ml)	0.11 ± 0.01	0.21 ± 0.01	0.14 ± 0.01	0.11 ± 0.01	0.10 ± 0.02	0.15 ± 0.01
		0.27 ± 0.00	0.42 ± 0.02	0.31 ± 0.00	0.20 ± 0.03	0.18 ± 0.01	0.28 ± 0.01
	DRE (7 ml)	0.10 ± 0.01	0.19 ± 0.01	0.12 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.13 ± 0.01
		0.24 ± 0.01	0.39 ± 0.02	0.21 ± 0.02	0.18 ± 0.01	0.18 ± 0.02	0.26 ± 0.01
	FOL (5 ml)	0.13 ± 0.01	0.21 ± 0.01	0.15 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.15 ± 0.00
		0.28 ± 0.03	0.44 ± 0.03	0.32 ± 0.05	0.24 ± 0.02	0.20 ± 0.03	0.29 ± 0.02
	FOL (7 ml)	0.12 ± 0.01	0.20 ± 0.01	0.14 ± 0.01	0.11 ± 0.02	0.11 ± 0.01	0.14 ± 0.01
		0.27 ± 0.03	0.42 ± 0.01	0.30 ± 0.03	0.21 ± 0.01	0.18 ± 0.01	0.27 ± 0.02
	Streptomycin	0.17 ± 0.01	0.20 ± 0.01	0.07 ± 0.01	0.11 ± 0.01	0.07 ± 0.01	0.9 ± 0.01
		0.33 ± 0.04	0.41 ± 0.02	0.14 ± 0.01	0.20 ± 0.01	0.14 ± 0.01	0.42 ± 0.03
	Ampicillin	0.18 ± 0.03	0.12 ± 0.01	0.09 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.24 ± 0.03
		0.30 ± 0.00	0.21 ± 0.01	0.18 ± 0.01	0.19± 0.02	0.21 ± 0.01	0.41 ± 0.01

Table 5
The mineral composition of dried SWE.

Composition	Ratio
Nitrogen (N)	0.4 ± 0.08
Phosphate (P_2O_5)	0.17 ± 0.01
Soluble potash (K_2O)	0.6 ± 0.1
Magnesium (Mg)	0.1 ± 0.02
Calcium (Ca)	0.1 ± 0.02
Sodium (Na)	0.9 ± 0.1
Iron (Fe)	$5 \times 10^{-4} \pm 1 \times 10^{-5}$
Copper (Cu)	$4 \times 10^{-5} \pm 1 \times 10^{-6}$
Zink (Zn)	$1 \times 10^{-4} \pm 1 \times 10^{-5}$
Manganese (Mn)	$9 \times 10^{-5} \pm 1 \times 10^{-6}$
Boron (B)	$3 \times 10^{-4} \pm 1 \times 10^{-5}$

Table 6
Carbohydrate composition in dried SWE.

Identified sugars	nmol/mg dried sample	Percentage (%)
Fucose	231.2 ± 3.1	20.3 ± 0.2
Xylose	205.3 ± 3.3	13.6 ± 0.1
Glucuronic acid	38.1 ± 1.1	5.1 ± 0.1
Mannitol	285.1 ± 3.2	29.8 ± 0.2
Mannose	87.9 ± 2.1	5.1 ± 0.1
Galactose	55.6 ± 1.4	5.0 ± 0.1
Glucose	113.3 ± 2.1	13.0 ± 0.1
Mannuronic acid	77.3 ± 1.7	8.1 ± 0.1

tially toxic ingredients (Skalicka-Woźniak and Walasek, 2014). Chouliaras et al. (2009) reported that *A. nodosum* SWE joint application with nitrogen and boron increased oil yield and enhanced the quality of the oil of olive trees by means of increasing the linolenic, as well as oleic acid concentrations. In the current study, the increase in essential oil content and the modified chemical composition might be attributed to several factors such as the presence of major and trace elements, as well as secondary metabolite elicitors in *A. nodosum* extracts. In the composition analysis of *A. nodosum* SWE, there were major elements such as nitrogen and phosphorus and trace elements including zinc and boron that may stimulate the growth and enrich the composition of treated plants. The presence of nitrogen and phosphorus had been correlated with increased oil production and improvement of the quality of the essential oils in several medicinal plants (Singh et al., 2002; Anwar et al., 2005; Sotiropoulou and Karamanos, 2010; Chrysargyris et al., 2016). Trace elements such as boron has been associated with carbohydrate metabolism and hormone functions in plants (Howe, 1998; Dordas and Brown, 2005). Misra and Sharma (1991) reported that zinc concentration in the nutrient application was critical for oil yield and menthol concentration in *Mentha arvensis* L.

A. nodosum is a brown macroalgae that is composed of polysaccharides including alginates, fucans, laminarin and carrageenans that play an important role in activating signaling pathways of salicylic acid, jasmonic acid and ethylene in plants (Vera et al., 2012). In addition, Rayorath et al. (2008a) reported that *A. nodosum* extracts modulate the accumulation and concentration of auxins in *Arabidopsis thaliana* and increased leaf numbers, root length, fresh weight compared to control plants, thus it may stimulate gibberellic acid independent amylase activity in barley (Rayorath et al., 2008b). Commercial SWE may contain macro- and micro-elements, vitamins, auxins, cytokinins and abscisic acid (ABA-Like) as well as betaines (Crouch and van Staden, 1993; Blunden et al., 1997; Stirk et al., 2004; Khan et al., 2009). It was reported in early studies that the metabolic elicitors are attached to receptor proteins on plant cell membranes, leading to increased production of secondary metabolites such as essential oils (Rayorath et al., 2008a; Vera et al., 2012; Bi et al., 2011; Sharma et al., 2014). Elicitors include polysaccharides such as alginic acid, laminarans (or laminarin) as well as carrageenans, main components of polysaccharides of several commercial seaweed liquid fertilizers including *A. nodosum*. Such carbohydrates had been associated with increased secondary metabolites in plants such as saponins, essential oils and phytoalexins (Aziz et al., 2003; Jeong and Park, 2005; Gururaj et al., 2012; Bi et al., 2011; Hashmi et al., 2012). Laminarin is composed of (1,3)-β-D-glucan with two types of chains ending with either mannitol or glucose residues and commonly available in *A. nodosum* extract and represents 5.8% of dry weight (Kadam et al., 2015). Fucoidan is

a polysaccharide found in *A. nodosum* and is mainly composed of fucose which had been found in this study in high ratio. Alginate is composed of mannuronic and guluronic acids with β (1, 4)-linkages in *A. nodosum* (Rioux et al., 2007; Jiang et al., 2010) and both mannuronic and guluronic acids had been found in the SWE used here. There was moderate ratio of carbohydrates within the dry matter, which indicates the presence of polysaccharides that may affect the physiological responses of the treated plants. In this study, the dose and methods of application had different effects on morphological and physiological parameters in two different species such as the drench application of SWE at 7 mL L^{-1}, which may agree with previous experiments performed using *A. nodosum* extracts on several crops and indicated that drench applications may have higher impact on plants (Spann and Little, 2011; Elansary et al., 2016).

The increased antibacterial activity of the essential oils of 7 mL L^{-1} SWE-treated mint plants might be attributed to the increase in L-menthone, L-menthol and α-terpineol in the respective plants. In a previous study by Mimica-Dukić et al. (2003), *M. piperita* showed the highest antibacterial activities against *Micrococcus flavus* which is in agreement with the current study. In addition, several reports on *M. piperita* showed that the strong antimicrobial activity of the essential oils against *Escherichia coli*, *B. subtilis* and *S. aureus* is attributed mainly to the menthol and menthone (Aflatuni et al., 2000; Işcan et al., 2002; Elansary and Ashmawy, 2013). The antibacterial activities of basil essential oil are in agreement with few reports showing medium to strong activities against some bacteria such as *E. coli*, *B. cereus* and *S. aureus* (Yavari et al., 2011; Azizkhani and Parsaeimehr, 2015). In basil plants, the essential oil of SWE-treated plants showed increases in the main oil constitutes such as linalool and chavicol methyl ether, which is responsible for the increased antibacterial activities found in the essential oil. The antibacterial activities of linalool as well as α-terpineol had been reported in several investigations (e.g. Queiroga et al., 2007; Mitić-Ćulafić et al., 2009; Park et al., 2012). The chavicol methyl ether as well as other compounds in the essential oil of basil had been associated with the antibacterial activities (Hossain et al., 2010; Yavari et al., 2011; Azizkhani and Parsaeimehr, 2015). The application of SWE on mint and basil plants enhanced the morphological and the physiological performance of the plants. Furthermore, it improved the medicinal value of the plants by increasing the content of the essential oils and enhancing the composition by means of main oil constitutes. SWE as biostimulants might be useful for enhancing mint and basil antimicrobial activities against wide-spectrum of bacteria.

5. Conclusion

A. nodosum extracts drench and foliar treatments enhanced most morphological parameters of leaf number and area, dry weights, and plant height of mint and basil plants compared to control plants. The increase in the essential oil content is associated with an increased oil composition by means of increased contents of L-menthone (32.4%) and L-menthol (32.6%) in mint SWE treated plants and increased chavicol methyl ether (38.7%), linalool (29.1%), and cineol (9.1%) in basil SWE treated plants. Additionally decreasing of potentially toxic pulegone and methofuran in mint oil was noticed. The stimulatory effect of *A. nodosum* SWE is largely attributed to available macro and micro nutrients, as well as carbohydrate contents. The higher antibacterial activity in SWE treated plants of mint and basil is associated with the increase of the main oil constitutes of their respective oils. In mint plants, the highest antibacterial activity found was in the essential oil of mint plants treated with drenched 7 mL L^{-1} SWE with MIC and MBC of 0.004–0.09 mg mL^{-1} and 0.010–0.19 mg mL^{-1}, respectively. The foliar application showed lower antibacterial activities compared to the drench application. In basil plants, the MIC of the essential oils ranged from 0.09 to 0.21 mg mL^{-1} whereas the MBC ranged from 0.18 to 0.44 mg mL^{-1}. The highest antibacterial activity found was in the essential oil of basil plants treated with drenched 7 mL L^{-1} SWE followed by both of the drench 5 mL L^{-1} SWE and foliar 7 mL L^{-1} SWE. The application of SWE as biostimulants for mint and basil plants may enhance their essential oils medicinal and pharmaceutical properties

Funding

The study was supported by the Biodiversity Institute of Ontario, University of Guelph and Henry Farms Canada.

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