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

Hosam O. Elansary[a,b,*], Kowiyou Yessoufou[c], Shadi Shokralla[a,d], Eman A. Mahmoud[e], Krystyna Skalicka-Woźniak[f]

[a] Biodiversity Institute of Ontario, University of Guelph, Guelph, Canada
[b] Floriculture, Ornamental Horticulture and Garden Design Department, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt
[c] Department of Environmental Sciences, University of South Africa, Florida campus, Florida, 1710, South Africa
[d] Department of Microbiology, Faculty of Pharmacy, Mansoura University, Egypt
[e] Department of Food Technology, Faculty of Agriculture, Damietta University, Egypt
[f] Department of Pharmacognosy with Medicinal Plants Unit, Medical University of Lublin, 1 Chodzki Str, 20-093 Lublin, Poland

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-

* Corresponding author at: Floriculture, Ornamental Horticulture and Garden Design Department, Faculty of Agricultue (El-shatby), Alexandria University, Egypt.
E-mail addresses: hoelansa@uoguelph.ca, hosammail2003@yahoo.com (H.O. Elansary).

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