Industrial Crops and Products 33 (2011) 704–709

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Effect of residual distillation water of 15 plants and three plant hormones on Scotch spearmint (*Mentha* × *gracilis* Sole)

Valtcho D. Zheljazkov[a,b,*], Tess Astatkie[c]

[a] *Mississippi State University, North Mississippi Research and Extension Center, 5421 Highway 145 South, Verona, MS 38879, USA*
[b] *University of Wyoming, Sheridan Research and Extension Center, 663 Wyarno Road, Sheridan, WY 82801, USA*
[c] *Department of Engineering, Nova Scotia Agricultural College, 50 Pictou Road, P.O. Box 550, Truro, NS B2N 5E3, Canada*

ARTICLE INFO

Article history:
Received 13 August 2010
Received in revised form 9 January 2011
Accepted 10 January 2011
Available online 2 February 2011

Keywords:
Distillation waste water
Plant hormones
Essential oil
Scotch spearmint

ABSTRACT

Distillation waste water is a byproduct from steam distillation of aromatic crops, and is currently discharged into streams and rivers. We evaluated distillation waste water (extract) from 15 essential oil crops plus three plant hormones (methyl jasmonate, MJ; gibberellic acid, GA3; and salicylic acid, SA) as foliar spray for Scotch spearmint (*Mentha* × *gracilis* Sole). GA3 and *Achillea millefolium* extract decreased essential oil content. *Hypericum perforatum* extract increased α-pinene, whereas SA decreased it. *H. perforatum* extract increased β-pinene and sabinene concentrations relative to hormones but was not different from the control. *H. perforatum* also increased L-limonene, while SA and GA3 decreased myrcene and MJ and SA decreased L-limonene. Application of MJ and SA increased L-carvone concentration relative to the control and most other treatments. The sulfur concentration in plant extracts was positively correlated to β-caryophyllene. None of the residual distillation waters showed significant antimicrobial or antimalarial activity. The distillation waste water from essential oil crops may serve as a modifier for Scotch spearmint essential oil.

1. Introduction

During the extraction of essential oils from plant biomass through steam distillation, four products are generated: (1) distillation waste water, (2) hydrolat, (3) essential oil, and (4) the steam-distilled (residual) plant material. The distillation waste water at the bottom of the container results from condensation of some of the hot steam passing through the plant biomass. The hydrolat is the distilled water that was separated from the essential oil and may contain some essential oil or water-soluble fractions of the distilled secondary metabolites. Hence, the hydrolat is usually redistilled to recover trace essential oil and other secondary metabolites (Lawrence, 2007; Topalov, 1962). The essential oil is the high-value product, but the residual plant material of most plant species is also valuable as animal feed (Djouvinov et al., 1997; Topalov, 1962). Currently, the distillation waste water is not a usable product and is released into the environment as a waste product. We found only one study on the use of distillation waste water as potential antioxidant and marinade ingredient to inhibit the development of undesirable flavor in turkey meat (Mielnik et al., 2008). There have been no reports on potential uses of the distillation waste water from various essential oil crops as growth promoters for plants. The objectives of this study were to evaluate the distillation waste water from 15 essential oil crops as foliar spray for another essential oil crop, Scotch spearmint (*Mentha* × *gracilis*) and to compare the results with the effects of three plant hormones (plant growth regulators, methyl jasmonate, MJ; gibberellic acid, GA3; and salicylic acid, SA).

Scotch spearmint (*M.* × *gracilis*) and Native spearmint (*Mentha spicata* L.) are grown as essential oil crops in the Midwest and northwestern United States (Lawrence, 2007; MIRC, 2009), with yearly production of over 1.0 million kg of oil (NASS, 2009). Recently, it was demonstrated that spearmints could be viable essential oil crops in the southeastern United States as well (Zheljazkov et al., 2010a,b). If a growth-promoting effect of the distillation waste water from any of the 15 essential oil crops is found, it could be used over a large production area of spearmint crops.

2. Materials and methods

2.1. Field production of 15 essential oil crops

To minimize environmental effects on the extracts from different species, the 15 essential oil crops were grown at an experimental field of the North Mississippi Research and Exten-

Abbreviations: MJ, methyl jasmonate; GA3, gibberellic acid; SA, salicylic acid.

* Corresponding author at: University of Wyoming, Sheridan Research and Extension Center, 663 Wyarno Road, Sheridan, WY 82801, USA. Tel.: +1 307 737 2415.
E-mail address: Valtcho.pubs@gmail.com (V.D. Zheljazkov).

0926-6690/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.indcrop.2011.01.011

sion Center at Verona in a randomized complete block design with four blocks. The essential oil crops were: wormwood, *Artemisia absinthium* L.; bishop's weed, *Ammi majus* L.; yarrow, *Achillea millefolium* L.; alecost, *Chrysanthemum balsamita* L.; lemon grass, *Cymbopogon flexuosus* (Nees ex Steud.) Will. Watson; palmarosa, *Cymbopogon martinii* (Roxb.) Wats.; hyssop, *Hyssopus officinalis* L.; St. John's wort, *Hypericum perforatum* L.; lavender, *Lavandula vera* D.C.; wild bergamot, *Monarda fistulosa* L.; shiso, *Perilla frutescens* (L.) Britton.; rue, *Ruta graveolens* L.; endemic Balkan winter savory, *Satureja pilosa* L.; Balkan sideritis, *Sideritis scardica* Griseb.; and feverfew, *Tanacetum parthenium* (L.) Sch. Bip. Seedlings from these crops were produced in a greenhouse using certified seeds and transplanted into the field after 40 days. The 15 crops were grown in raised beds covered with black plastic mulch and irrigated through a drip-tape irrigation system placed under the plastic and 2- to 3-cm soil depth. All 15 crops were grown under the same water and fertilizer regime and harvested at flowering, which corresponds to the technical maturity of these crops for essential oil production (Topalov, 1962; Zheljazkov, 1998). Plants were harvested at approximately 10 cm above the soil surface and were dried in a shady well-aerated location with temperature up to 40 °C to preserve essential oil and minimize oil losses (Lawrence, 2007; Topalov, 1989).

Representative samples (approximately 300 g of dried biomass) from each of the 15 essential oil crops were steam distilled for 60 min in 2-L Clevenger type distillation units (Zheljazkov et al., 2010a). All 15 crops were distilled in the same distillation apparatuses, under the same distillation conditions, and in three replicates. The residual distillation water from each plant species was collected at the end of each distillation and kept refrigerated until used for foliar spray.

2.2. Plant materials and growing conditions for the container experiment

Scotch spearmint (*M.* × *gracilis* Sole) was used in this study. To avoid plant disease and pest issues, the experiment was conducted with certified and virus-free planting material purchased from the Summit Plant Laboratories, Inc. (Fort Collins, CO). The plants were received in plastic trays and were 10–12 cm high, with well-developed roots and several pairs of leaves. All commercial varieties of spearmint and peppermint are propagated exclusively vegetatively, due to the fact they are either hybrids or sterile (Lawrence, 2007; Tucker and Fairbrothers, 1990; Tucker, 1992).

After hardening for a week, spearmint plants were transplanted into 3-gallon pots (two plants per pot), filled with 3.1 kg of commercial growth medium (Metromix 300, Sun Gro Horticulture, Bellevue, WA). Plants were grown from May to August 2007 in a controlled-environment greenhouse, with day temperatures of 22–25 °C and night temperatures of 18–20 °C. Water was provided using an automatic irrigation system with individual drip-type emitters for every pot. The total amount of fertilizers applied to each pot was equivalent to 240 kg N/ha under field conditions. These were applied using the controlled-release fertilizer CRF (Osmocote Plus 15N-9P-12K; Scotts-Sierra Horticultural Products Co., Marysville, OH) immediately after transplanting and with the water-soluble fertilizer WSF 100 mg/kg of N with 1100 mL of WSF (greenhouse grade NPK fertilizer 20N–8.8P–16.6K general purpose; Scotts-Sierra Horticultural Products Co.) applied every 2 weeks. Plants in each pot developed well; they did not exhibit any signs of nutrient deficiency and were not infested by diseases or pests.

The experiment had a completely randomized design with three replicates; pots were rotated on a weekly basis on the benches to avoid any potential localized effect on plants. Treatments 1–9 were the three plant hormones at various concentrations (MJ at 10, 100, and 1000 mg/L; GA3 at 10, 100, and 1000 mg/L; and SA at 10, 100, and 1000 mg/L); treatments 10–24 were the residual distillation water (extracts) from 15 essential oil crops; and treatment 25 was the water control.

Spearmint plants were sprayed twice with hormone solutions and extracts: the first application was at the beginning of bud formation, and the second was at the beginning of flowering. Treatments were applied by spraying all aboveground plant parts in each pot using approximately 10 mL of solution per pot. Spearmint plant biomass from each pot was harvested 14 days after the second treatment, when plants were fully flowering, by cutting the aboveground herbage at approximately 5 cm above the growth medium. Commercial spearmint plantations are harvested at flowering to obtain the highest essential oil yields with desirable oil composition (Lawrence, 2007; Topalov, 1989). Fresh herbage yields were recorded immediately and dry herbage yields were recorded after drying at temperatures of 35–40 °C in a shady location.

2.3. Essential oil extraction and gas chromatography analysis of the spearmint oils

The spearmint oil from the dried plant biomass from each pot was extracted using 2-L steam distillation apparatuses for 60 min, as described previously (Zheljazkov et al., 2010a). Spearmint oil was collected at the end of each distillation by separation from water, the oil was weighed, and the essential oil content in dried spearmint herbage was calculated as the amount (g) of oil per weight (g) of dry herbage. Spearmint oil samples were kept at −5 °C until the gas chromatography analysis.

The essential oil analyses were conducted on a Hewlett Packard 6890 gas chromatograph with an auto sampler (carrier gas helium, 40 cm/s; 11.7 psi [60 °C]; 2.5 mL/min constant flow rate; injection: split [60:1], 0.5 μL, inlet 220 °C; oven temperature program: 60 °C for 1 min, 10 °C/min to 250 °C; column: HP-INNOWAX [cross-linked PEG], 30 m × 0.32 mm × 0.5 μm; FID detector temperature 275 °C).

2.4. Assay for in vitro antileishmanial, antimicrobial and antimalarial activity

The residual distillation waters (extracts from the 15 essential oil crops used in this study) were screened for antileishmanial activity *in vitro* on a culture of *Leishmania donovani* promastigotes using the assay of Mikus and Steverding (2000). Antimicrobial and antimalarial activity of the distillation waters were evaluated at concentrations up to 50 μg mL^{-1} and as described previously (Bharate et al., 2007).

2.5. Measurement of nutrients in residual distillation waters

The concentration of plant nutrients and trace elements in the distillation waters was measured on inductively coupled argon plasma spectrometer (ICAP) (Thermo Jarrell Ash, Franklin, MA) at the Mississippi State Soil Testing Laboratory, using commonly accepted methods (Cox, 2001). The concentration of nitrate-N in the residual distillation waters was measured using a nitrate electrode (Francis and Malone, 1975), at the Mississippi State Soil Testing Laboratory.

2.6. Statistical analysis

The 14 biomass and constituent response measurements were analyzed as a completely randomized design with 25 treatments. The analysis of variance (ANOVA) was performed using the GLM Procedure of SAS (SAS, 2008), and further multiple means comparison was performed when the treatment effect was significant (p-value < 0.05) using Duncan's multiple range test. Duncan's multiple means comparison method was used as a compromise