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Responses of *Myzus persicae* (Sulzer) to three Lamiaceae essential oils obtained by microwave-assisted and conventional hydrodistillation

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ABSTRACT

The essential oils derived from three plants within the Lamiaceae family, *Origanum majorana* L. (marjoram), *Mentha pulegium* L. (pennyroyal), and *Melissa officinalis* L. (lemon balm), using two techniques for their isolation, were evaluated for their effects on longevity and fecundity of the aphid *Myzus persicae* (Sulzer). The two techniques employed were Clevenger hydrodistillation (i) using conventional heating (HD) and (ii) assisted by microwaves (MWHD). Analysis of the essential oils by GC-MS showed an increase in the oxygenated fraction determined in MWHD oils. The effects of essential oils were screened with two concentrations, 250 and 500 μl/l, on aphid adults <24 h old enclosed individually in clip-cages on eggplants (*Solanum melongena* L.). All the essential oils significantly reduced aphid longevity and fecundity, compared with the controls. *M. pulegium* oils exhibited the most significant activity at 500 μl/l, with longevity shortened by 8 days (87% reduction) and fecundity reduced more than 40-fold. Carvacrol and piperitone were the main constituents of the *O. majorana* and *M. pulegium* essential oils, respectively. Their toxic action was shown to be similar to that of the respective essential oils. Mixtures of commercially purchased pure compounds (citral, β-caryophyllene, caryophyllene oxide) associated with *M. officinalis* essential oils were also proved effective. These results indicate a high potential of *O. majorana*, *M. pulegium*, and *M. officinalis* essential oils but also of their major constituents as alternatives in the management of *M. persicae*. However, further studies should follow to test the bioefficacy of these oils in larger scale experiments. The comparison of the efficacy of essential oils produced with the traditional HD and the MWHD technique showed no significant difference. Thus, the use of MWHD for the extraction of essential oils for pest control is suggested, considering also its well-known advantages such as shorter distillation time and less energy consumption.

1. Introduction

Essential oils are plant-derived secondary metabolites that play an important role in the protection of plants as antibacterials, antivirals, antifungals, or insecticides. In integrated pest management, these complex natural mixtures have a promising potential in the effort to substitute environmentally compatible pest-control alternatives for synthetic pesticides (Isman, 2008, 2000; Shaaya and Rafaeli, 2007).

The potential of essential oils in pest management has been proved beneficial against Coleoptera, Homoptera, mites, and Lepidoptera (Isman, 2000). Among the target pests, aphids (Homoptera: Aphididae), and especially *Myzus persicae* (Sulzer) which is one of the most common aphid pests worldwide, have attracted considerable attention due to their importance as crop pests along with their ability to develop resistance to insecticides (Criniti et al., 2008; Fuentes-Contreras et al., 2013; Srigiriraju et al., 2010). Antifeedant effects on *M. persicae* were found for the essential oils obtained from Argentinean *Baccharis salicifolia* (Ruiz & Pav.) Pers. and *Eupatorium* spp. as well as from Spanish *Artemisia absinthium* L. (Asteraceae) (Bailen et al., 2013; Sosa et al., 2012). In olfactometer studies, essential oil of *Hemizygia petiolata* Ashby (Lamiaceae) had a repellent

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effect against two aphid pests, but *M. persicae* was not affected (Bruce et al., 2005).

In the search for bioactive compounds, research efforts have recently focused on the exploitation of native/endemic plants. Plants originating in the Mediterranean region have attracted scientific interest owing to the high variation of the regional microclimate and the conspicuous biodiversity. In fact, essential oil composition and yield can vary within a plant species depending on the climatic conditions, the soil, or other characteristics of the areas where it is grown (Kokkini et al., 2004; Silvestre et al., 1997; Vokou et al., 1993).

Among the plant-derived essential oils, those within the Lamiaceae family have received considerable attention (Isman, 2000; Koschier and Sedy, 2003; Saroukolai et al., 2010). In a detailed study, Sampson et al. (2005) showed that adults of turnip aphid *Lipaphis pseudobrassicae* (Davis) were susceptible to essential oils including *Satureja thymbra* L. (Lamiaceae). The *Origanum majorana* L. (marjoram) and *Mentha pulegium* L. (pennyroyal) (Lamiaceae) essential oils inhibited feeding and settling but showed no repellent activity, while pennyroyal oil directly affected survival of *M. persicae*, as a result of its oral and/or fumigant toxicity (Hori, 1999). However, no chemical composition of both oils was provided. Therefore, marjoram and pennyroyal essential oils may affect survival or reproduction of *M. persicae*, but this potential has not yet been explored. To our knowledge, the effects of the essential oil derived from *Melissa officinalis* L. (Lamiaceae) (lemon balm) on aphid pests, in general, have not yet been investigated as well.

The essential oils of aromatic plants are usually obtained by hydrodistillation or steam distillation. Alternative methods, employing microwaves, have been developed in order to shorten the time required, decrease the solvent consumption, increase the yield, and enhance the quality of isolated oil (Bendahou et al., 2008; Ferhat et al., 2006; Lucchesi et al., 2004). Microwave energy, a non-contact heat source, offers more effective and selective heating, faster energy transfer, reduced thermal gradients, faster response to process heating control, and faster start-up than traditional heating techniques (Ferhat et al., 2006). It has been documented that application of microwaves results in essential oils with higher amounts of oxygenated constituents (Ferhat et al., 2006; Lucchesi et al., 2004; Riela et al., 2008), compared with the use of conventional heating. This is important considering that oxygenated compounds are generally more toxic to insect pests than hydrocarbons (Papachristos et al., 2004; Tomova et al., 2005).

Another major issue aiming to support the industrial development of new pesticides based on essential oils is to search whether the major compounds of the essential oil under study have, and to what extent, similar insecticidal identities to the essential oil itself (Hummelbrunner and Isman, 2001; Isman et al., 2011).

Based on the above considerations, the objectives of the present study were (a) to investigate the effects of essential oils derived from three selected Lamiaceae plants, *O. majorana*, *M. pulegium*, and *M. officinalis*, on *M. persicae* longevity and fecundity; (b) to test the bioactivity of the major compound(s) of each essential oil and compare with the bioactivity of each respective essential oil; and (c) to compare the two heating techniques used for the essential oil extraction, i.e. hydrodistillation (HD) and microwave-assisted hydrodistillation (MWHD), in terms of isolation time, yield, chemical composition, and the comparative bioactivity of essential oils.

2. Materials and methods

2.1. Plant materials and chemicals

The aerial parts (leaves, flowers) of *O. majorana* and *M. pulegium* were collected from local fields in Attiki (central Greece) and Orestiada (north-eastern Greece), respectively, during the flowering period (July–August). Leaves of *M. officinalis*, collected in August, were provided by a local producer in Agrinio (western Greece). All samples were air-dried and stocked until distillation. Carvacrol, citral, citronellol, β-ionone, β-myrcene, α-terpinene, thymol, α-terpineol, limonene, β-caryophyllene, γ-terpinene, citronellal, linalool and nerol were purchased from Sigma (St. Louis, USA). Menthone, α-pinene, β-pinene, *p*-cymene and geraniol were obtained from Aldrich (Steinheim, Germany). Linalyl acetate, sabinene hydrate, caryophyllene oxide, terpinen-4-ol, α-phellandrene and β-ocimene were purchased from Fluka (Steinheim, Germany). Terpinolene, *iso*-menthone and piperitone were acquired from Extrasynthese (Genay, France). Neryl acetate was obtained from Alfa Aesar (Karlsruhe, Germany). All authentic compounds used have an average purity of 95%. Diethyl ether (BHT-free) and pentane were acquired from SDS (Peypin, France) and Lab-Scan (Dublin, Ireland), respectively. Silica Gel 60G and TLC plates (silica gel 60, F_{254}) were purchased from Merck (Darmstadt, Germany).

2.2. Isolation of essential oils

A sample (200 g) of the air-dried material of each of the three tested plants was ground in an electrical blender and then divided into two parts of 100 g. Each part was submitted separately to hydrodistillation in a Clevenger-type apparatus employing microwave-assisted hydrodistillation (MWHD) or hydrodistillation technique (HD) as described by Stashenko et al. (2004) with slight modifications. MWHD was conducted in a modified domestic microwave oven (LG, 700 W) with a top orifice through which an external glass condenser joined a round flask, with the plant material (100 g) and deionized water (500 ml), inside the oven. The distillation time was 30 min at full power, until no more essential oil was obtained. HD was performed in a similar manner but using a heating mantle for 4 h, instead of microwave irradiation. The whole procedure was conducted in triplicate for each of the three plants. Essential oils, decanted from hydrosols, were dried over anhydrous magnesium sulfate. After filtration, volume was determined and expressed as ml of essential oil/100 g of dry material (Table 1) and the oils were stored in labelled sterile screw capped bottles at −22 °C.

2.3. Analysis of essential oils

Gas chromatography (GC) analyses were conducted using a Hewlett-Packard 5890 II GC-FID system, equipped with an HP-5ms capillary column (30 m × 0.25 mm, film thickness 0.25 μm). The injector and detector temperature were 220 °C and 290 °C, respectively. Experimental conditions induced an oven temperature GC programmed from 60 to 240 °C at a rate of 3 °C/min and then held isothermally for 10 min. The carrier gas was helium, at a flow rate of 1 ml/min. Diluted samples of 1.0 μl (1/100 in diethyl ether, v/v) were injected manually in splitless mode. Quantitative data were obtained electronically from FID area percent data without the use of correction factors. Qualitative analysis of the essential oils was performed under the same conditions with GC, using GC coupled with a Hewlett-Packard 5972 mass selective detector, operating in EI mode at 70 eV. The retention indices (RI) of compounds were determined relative to the retention times of *n*-alkanes (C_8–C_{20}) with linear interpolation, using the Van Den Dool and Kratz equation (Van Den Dool and Kratz, 1963). Identification of the major essential oil components was based on the comparison of their retention indices with those of authentic compounds by coelution and MS analysis. For the other components, tentative identification involved matching retention indices and recorded mass spectra