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Chemical composition and biological activities of *Laurus* essential oils from different Macaronesian Islands

R. Furtado [a], J. Baptista [b], E. Lima [b], L. Paiva [b], J.G. Barroso [c], J.S. Rosa [d], L. Oliveira [e,*]

[a] Departamento de Biologia, Universidade dos Açores, Rua da Mãe de Deus, 9501-801 Ponta Delgada, S. Miguel, Açores, Portugal
[b] CITA-A, CIRN, Departamento de Ciências Tecnológicas e Desenvolvimento, Universidade dos Açores, Rua da Mãe de Deus, 9501-801 Ponta Delgada, S. Miguel, Açores, Portugal
[c] Universidade de Lisboa, Faculdade de Ciências de Lisboa, DBV, IBB, Centro de Biotecnologia Vegetal, C2, Campo Grande, 1749-016 Lisboa, Portugal
[d] CIBIO, InBIO Laboratório Associado, Pólo dos Açores, Departamento de Biologia da Universidade dos Açores, Rua da Mãe de Deus, 9501-801 Ponta Delgada, S. Miguel, Açores, Portugal
[e] CBA-IBB, CIRN, Departamento de Biologia, Universidade dos Açores, Rua da Mãe de Deus, 9501-801 Ponta Delgada, S. Miguel, Açores, Portugal

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ABSTRACT

Essential oils (EO) and eight pure components from the fresh leaves of *Laurus novocanariensis* (LN) from Madeira and of *Laurus azorica* (LA) from four Azorean Islands (S. Miguel-SMI, S. Maria-SMA, Pico-PIC and S. Jorge-SJO) were evaluated for fumigant and contact insecticidal effects on adult stage of Mediterranean fruit fly (medfly) and for inhibition of oviposition. The chemical composition of EO were analysed by GC/MS. Oxygen-containing mono- and sesquiterpenes (mainly 1,8-cineole) dominated in LN (50%), LA-SMA (88%) and LA-SMI (57%) and mono- and sesquiterpene hydrocarbons (mainly α-pinene) dominated in LA-PIC (61%) and LA-SJO (44%). Linalool and α-terpinyl acetate were also abundant in LA-SMA and *trans*-cinnamyl acetate in LA-SJO. These three components and all of the *Laurus*' EO showed high repulsive activity against medfly oviposition. In contact assays on medfly adults, a moderate degree of mortality was observed, being the most toxic samples, in decreasing order, *trans*-cinnamyl acetate > α-terpinyl acetate > LA-SMI ~ LA-SMA > LA-SJO ~ linalool. In fumigant assays, α-terpinyl acetate was the only compound that showed some toxicity on medfly adults. According to the obtained results, the *Laurus* EO seem promising to be used against medfly oviposition in integrated pest management strategy.

1. Introduction

Most species of the Lauraceae possess aromatic roots, stems and fruits. Some popular spices originated from this family, such as the Mediterranean Bay laurel (*Laurus nobilis* L.), are of economic value. The genus *Laurus* includes two other important species, the Macaronesian *Laurus azorica* (Seub.) Franco (native of Azores Archipelago) and *Laurus novocanariensis* Rivas-

* Corresponding author. Tel.: +351 296 650 116; fax: +351 296 650 100.
E-mail address: ziza@uac.pt (L. Oliveira).

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Mart., Lousa, Fern. Prieto, E. Días, J.C. Costa and C. Aguilar (native of Madeira Island and Canary Islands). The diagnostic key characters of these three species often overlap (Rodilla et al., 2008; Rodríguez-Sánchez et al., 2009). Previous works on *Laurus* species have reported the essential oils (EO) chemical composition according to the location and phenological stage (Pedro et al., 2001; Rodilla et al., 2008), as well as antimicrobial, antioxidant, molluscicidal and insecticides properties (Conforti et al., 2006; Rodilla et al., 2008; Rosa et al., 2010; Teixeira et al., 2012).

Ceratitis capitata (Wiedemann) (Diptera: Tephritidae), medfly, is one of the world's most economically damaging pest species (Beroiz et al., 2012). In the last few years, the development of crop protection methods has taken into account the need to preserve the precarious equilibrium between organisms and environmental conditions (Passino et al., 1999). Insecticides such as organophosphates are currently the principal way of controlling this pest. Traditional biological control is also a possible way for fighting against medfly, with the application of parasitoids, predators or the use of microbiological control agents. Another promising complement or alternative strategy for this insect control can be the use of plants extracts or allelochemicals such as EO (Benelli et al., 2012).

The aim of this study was (1) to assess the chemical polymorphism of the EO isolated from winter leaves of *L. azorica* populations growing on different Azores Islands and of *L. novocanariensis* from Madeira Island, and (2) to determine the insecticidal properties of these oils and some of its components on medfly adult's mortality by contact or fumigation assays, as well as their oviposition repellency, under the laboratory conditions.

2. Material and methods

2.1. Plant material and essential oils extraction

Fresh leaves of *L. azorica* were collected from the laurel-juniper forest at four different Islands of the Azores: São Miguel (SMI), Santa Maria (SMA), São Jorge (SJO) and Pico (PIC) and fresh leaves of *L. novocanariensis* were collected from the laurel forest of Madeira (MAD) Island, during the winter (2011). Leaves, randomly picked from healthy plants during the vegetative period were placed in plastic bags and immediately brought to the laboratory and stored at −20 °C. The leaves were defrosted at room temperature and dried under dark conditions in an oven chamber during 3 days at 25 °C (to avoid bacteriological and fungal contaminations) before hydrodistillation. Taxonomic identification of plants was performed by botanists of Herbarium of Department of Biology, Azores University. Voucher specimens were deposited at the Department of Technological Science and Development, University of Azores (voucher numbers UA-DCTD178 for *L. novocanariensis* and UA-DCTD 179-182 for *L. azorica* from SMI, SMA, SJO and PIC Islands, respectively). The EO were extracted from the dried leaves (500 g) by hydrodistillation for 2 h in a Clevenger-type apparatus. The isolated EO were dried over anhydrous sodium sulphate and stored at 4 °C in dark prior to analysis.

2.2. Chemicals

The pure components α-pinene, β-pinene, 1,8-cineole, γ-terpinene, linalool, limonene, α-terpinyl acetate and *trans*-cinnamyl acetate, used for the bioassays, were purchased from Sigma Chemical (St. Louis, MO, USA).

2.3. Essential oils analysis

EO were analysed using a Claurus GC system model 600 equipped with a DB-1 (30 m × 0.25 mm ID, 0.25 μm film thickness) column (J&W Scientific Inc.), hyphenated with a mass spectrometer Perkin–Elmer Turbomass Clarus 600T (software version 5.4). The analytical conditions were: oven temperature from 45 °C to 175 °C at a rate of 3 °C min^{-1} followed by a temperature increase at 15 °C min^{-1} until 300 °C and hold during 10 min; transfer line and ion source temperatures, 280 °C and 220 °C, respectively; carrier gas Helium at a linear velocity of 30 cm s^{-1} at 45 °C; split ratio, 1:40; electron impact (EI) mode at 70 eV; ionization current of 60 μA; mass scan range of 40–300 amu, and scan time of 1 s. The identity of the EO components was assigned by comparison of their retention indices, relative to C9–C17 *n*-alkanes, and GC–MS spectra with corresponding data of (1) components of reference oils: *Thymus caespititius* (Salgueiro et al., 1997), *L. azorica* (Pedro et al., 2001), *Coriandrum sativum*, *Satureja montana*, *Santolina chamaecyparissus*, *Thymus vulgaris* (Grosso et al., 2010) and *Monizia edulis* (Figueiredo et al., 1997), (2) laboratory-synthesized components following the methods described by Grosso et al. (2010) and (3) commercially available standards from a home-made library. For quantification, their raw percentage was calculated by integration of TIC Chromatogram peaks without correction factors.

2.4. Insects

C. capitata were obtained from laboratory colony, maintained for >20 generations under laboratory conditions with a 14L:10D photoperiod, 70 ± 5% humidity and 24 ± 1 °C temperature. Larvae were reared with an artificial diet, according to the methodology described by Albajes and Santiago-Álvarez (1980) and adults were raised with a mixture of yeast hydrolysate (FLUKA Analytical, Spain) and sugar (1:3 w/w), and water as a supplement.