Industrial Crops and Products 84 (2016) 418–422

Contents lists available at ScienceDirect

Industrial Crops and Products

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

The essential oil of *Laurelia sempervirens* is toxic to *Trialeurodes vaporariorum* and *Encarsia formosa*

Nelson Zapata[a,*], Marisol Vargas[a], Esteban Latorre[a], Ximena Roudergue[a], Ricardo Ceballos[b]

[a] Faculty of Agronomy, University of Concepcion, Av. Vicente Méndez 595, Chillán, Chile
[b] Agricultural Research Institute, Av. Vicente Méndez 515, Chillán, Chile

ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form 5 February 2016
Accepted 11 February 2016
Available online 26 February 2016

Keywords:
Essential oils
Laurelia sempervirens
Botanical insecticides
Trialeurodes vaporariorum
Encarsia formosa

ABSTRACT

Essential oils can be an important tool for controlling crop pests, but to use it as a complement of biological control is also necessary to determine its toxicity to beneficial insects. The goal of this research was to study the fumigant activity of essential oil (EO) extracted from leaves of *Laurelia sempervirens* R. et. P. Tul. against adults of *Trialeurodes vaporariorum* (Westwood) and the parasitoid *Encarsia formosa* (Gahan) under laboratory conditions. The compounds found in higher percentage in the EO were: safrole (α and β isosafrole) (33.9%), linalool (16.18%), and α-pinene (8.55%). The EO (1–16 $\mu L\,L^{-1}$ air) was very effective against *T. vaporariorum* (LC_{50} = 3.77 $\mu L\,L^{-1}$ air), but also very toxic for *E. Formosa* (LC_{50} = 0.86 $\mu L\,L^{-1}$ air). The essential oil worked faster at higher concentrations, and when the EO was assayed at concentration from 2 to 16 $\mu L\,L^{-1}$ air, the LT_{50} values dropped from 4.27 h to 2.61 h for *T. vaporariorum*, and from 4.04 h to 0.21 h for *E. Formosa*. The reproduction of *E. formosa* was significantly affected by the EO when applied at very low doses. The host attack percentage was lower, and the number of descendants per female also decreased. These preliminary results indicate that it would not be possible to use this EO in combination with *E. formosa* for controlling the greenhouse whitefly *T. vaporariorum*.

1. Introduction

The greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) is one of the most important agricultural pests worldwide, and is hosted by more than 250 plant species (Hilje and Morales, 2008). In Chile, this whitefly affects a wide range of crops (Prado, 1991), but the most affected is the tomato grown in greenhouses (Vargas and Alvear, 2000). Whitefly directly damages crops by sucking sap, which causes a reduction in the plants' vitality and affects their productive potential. The honeydew that the flies produce when feeding is a growth substrate for sooty mold, which fouls the leaves and fruit, affecting physiological processes in the plant (Hilje and Morales, 2008). They can also transmit viruses to their hosts (Jones, 2003).

To control this whitefly, chemical and biological methods can be used, although it would be best to perform an integrated management of this pest (Gorman et al., 2002). Chemical control involves the use of synthetic insecticides, but the resistance developed by the whitefly to these pesticides forces producers to make repeated treatments, not always with satisfactory results (Capinera, 2008), and also causing unwanted side effects such as reducing the number of pollinating insects and the natural enemies of whitefly (Van Lenteren, 2000).

Biological control of Whitefly is mostly addressed by using the endoparasitoid encarsia *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae), which is widely distributed throughout the world (Liu et al., 2015). However, it should be considered that biological controllers are not fast acting, and as a single strategy can be more expensive than other methods (Bale et al., 2008). Moreover, it is not possible to simultaneously use the most commonly used insecticides for control of whitefies, such as abamectin, acetamiprid, imidacloprid, or deltametrin, without causing significant mortality of the parasitoid (Araya et al., 2006; Yankova et al., 2011). For these reasons, it is necessary to evaluate new alternatives for controlling this whitefly, strategies that can be ideally used in conjunction with natural enemies such as the parasitoid encarsia.

When analyzing options it is possible that essential oils could play an important role as alternative tools for controlling this pest. Previous studies on insecticidal activity of essential oils have been shown to be able to fully or partially replace synthetic insecticides

* Corresponding author. Fax: +56 42 275309.
E-mail address: nzapata@udec.cl (N. Zapata).

http://dx.doi.org/10.1016/j.indcrop.2016.02.030
0926-6690/

(Regnault-Roger, 1997; Isman, 2000; Choi et al., 2003; Yang et al., 2010). Product of their physical and chemical properties, essential oils can poison insects in different ways: through inhalation, given their diversity of volatile compounds; by contact, due to the formation of an impermeable film that suffocates; and by deep penetration due to the amphibole nature of some of their compounds (Regnault-Roger et al., 2004).

It is stated that essential oils have the advantage of being rapidly biodegradeable, generally exhibit low toxicity to beneficial insects, and do not cause imbalances in the ecosystem (Isman, 2000). However there are few studies with relation to its affects on the natural enemies of pests. Due to its varied composition, some essential oils could be equally toxic to pests and their natural enemies, which could limit its use in programs of integrated pest management. (Suthisut et al., 2011).

The essential oil extracted from the Chilean laurel *Laurelia sempervirens* R. et. P. Tul., (Atherospermataceae), a native Chilean tree plant, has proven to be a powerful natural insecticide when applied by contact and as a fumigant on stored grain pests and the pea aphid *Acyrthosiphon pisum* Harris. (Bittner et al., 2008; Zapata et al., 2010; Zapata and Smagghe, 2010). The Chilean laurel essential oil might be a good alternative for pest control in greenhouses, such as for whitefly. However, if one also wants to implement an integrated control strategy for this pest, where the vitality of the biological control should be considered, then it is crucial to investigate the toxicity of this essential oil for the parasitoid encarsia. Given this background, the goal of this research was to study the fumigant activity of an essential oil obtained from leaves of Chilean laurel against the greenhouse whitefly and the parasitoid encarsia.

2. Materials and methods

2.1. Plant material and oil extraction

Leaves of Chilean laurel were collected in October of 2012 in the South-Central Region of Chile (36°51′S, 71°57′W). Fresh plant material was washed with distilled water to remove any debris and then was crushed. Essential oil was extracted from the plant samples using a Clevenger-type apparatus, where the plant material is subjected to hydrodistillation for 4 h. Anhydrous sodium sulphate was used to remove water after extraction. Oil yield was calculated according to the fresh weight of the plant material. The extracted oils were stored in a refrigerator at 4 °C until use for the insect tests.

2.2. Analysis of essential oil composition

An SPME holder equipped with a 75-μm Carboxen/Polydimethylsiloxane fiber (Supelco Inc., Bellefonte, PA) was used, and prior to volatile collection the fiber was activated according to the manufacturer's instructions. For each extraction, 3 mL of the essential oil was transferred into a 15 mL amber vial, and to achieve the partition equilibration between the sample and headspace inside the vial a period of 1 h was permitted. Later the SPME fiber was exposed to the vial headspace to absorb the analytes for 2 h. The essential oil volatile-component analysis was performed by direct injection of the fiber in a gas chromatographer/mass spectrometer (GCMS QP2010 Plus; Shimadzu, Tokyo, Japan) equipped with a Restek capillary column (Rxi-5 ms: 5% diphenyl-95% dimethylpolysiloxane; 30 m × 0.25 mm ID × 1.0 μm; Restek Corporation, Bellefonte, Pennsylvania, USA). Helium gas was used as the gas carrier, with a flow rate of 1.4 mL min^{-1}. Mass spectrum acquisition was performed in the mass range from 35 to 500 *m*/*z*. The ionization was performed by electron impact at 70 eV with an ion source at 200 °C. The GC oven was programmed to remain at 40 °C for 5 min with subsequent increases of 5 °C min^{-1} until 230 °C, where it was held for 10 min. The interface temperature was programmed at 250 °C. The resulting spectra were compared with a library data-base by a reverse search technique using the National Institute of Standards and Technology (NIST) mass spectral search program (version 2.0; Standard Reference Data, NIST, Gaithersburg, Maryland, USA).

2.3. Insect rearing

Whiteflies were obtained from stock colonies maintained in the Laboratory of Phytochemistry, Faculty of Agronomy, at Concepcion University, Chile. The insects were reared on tomato plants which were grown in a bioclimatic chamber at 25 °C, with 65 ± 5% relative humidity, and a 16:8 (light:dark) photoperiod. When whiteflies of the same age were required, oviposition was allowed for 12 h on uncolonized tomato plants, then the adults were removed and the plants with eggs were kept in ventilated, plastic boxes, isolated from the rest of the population. At the same time, parasitoids of Encarsia were reared on a parallel population of whitefly, which was maintained under the same conditions as described above. When parasitoids of the same age were required; Nymphs (N3) of whiteflies were exposed to females of encarsia for 12 h. Subsequently, plants containing nymphs were kept in small, ventilated cages until emergence of the adults.

2.4. Fumigation chamber

In all bioassays, the essential oil was applied in a fumigation chamber, which consisted of a glass flask (500 mL) with a sealing lid. Inside the lid a filter paper disc of 2 cm diameter was attached, where essential oil was applied on this filter paper according to the desired concentration, and then covered with a small mesh cap to avoid direct contact with the insects. Environmental conditions for all bioassays were 20 ± 2 °C, 50% RH with a 16:8 (light:dark) photoperiod.

2.5. Dose–response bioassays

Dose–response assays were carried out with essential oil against whitefly (1.0–16 μL L^{-1} air) and encarsia (1.0–5.0 μL L^{-1} air) adults. Newly emerged adults (<24-h-old) were gently placed in fumigation chamber with the help of an entomological aspirator (20 insects/fumigation chamber). Each treatment was repeated four times, including a control in which only a drop of water was applied on the filter paper. The insects were fumigated for 4 h and the dead insects were counted in each unit. The insects were considered dead when they did not show any movement while being gently touched with a camel hair brush. LC_{50} and LC_{90} values were calculated according to Finney (1971).

2.6. Lethal time bioassays

Assays for determining lethal fumigation times were established for whitefly and encarsia. Concentrations of 2, 4, 8 and 16 μL of oil L^{-1} air were tested. A treatment without application of oil was also considered. As described above, twenty insects were introduced into the fumigation chamber. Each treatment was repeated four times. The insects were kept under fumigation for 10, 20, 40 min and 1, 2, 4, 8, 12 and 24 h periods. The dead insects were counted in each unit and the lethal times (LT_{50}, LT_{90}) were calculated (Finney, 1971).

2.7. Effect of essential oil on the reproduction of encarsia

To assess the effect of sub-lethal concentrations of the essential oil on the reproduction of the parasitoid, five females (48-h-old)