ARTICLE IN PRESS

Industrial Crops & Products xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Use of nanoemulsions of plant essential oils as aphid repellents

M.J. Pascual-Villalobos[a,*], M. Cantó-Tejero[a,b], R. Vallejo[a,c], P. Guirao[b], S. Rodríguez-Rojo[c], M.J. Cocero[c]

[a] Instituto Murciano Investigación y Desarrollo Agrario y Alimentario (IMIDA), C/Mayor s/n 30150 La Alberca, Murcia, Spain
[b] Universidad Miguel Hernández, E.P.S.Orihuela, Carretera de Beniel Km. 3,2, 03312 Orihuela, Alicante, Spain
[c] Universidad de Valladolid, Escuela de Ingenierías Industriales, Sede Dr. Mergelina, c/Doctor Mergelina s/n, 47011 Valladolid, Spain

ARTICLE INFO

Keywords:
Rhopalosiphum padi
Anise
trans-Anethole
Citral
Carvone
Caryophyllene
Farnesol
cis-Jasmone
cis-Hexenol
Geraniol
Mint
Lecithin
Emulsion
Formulation

ABSTRACT

It is believed that climate change will greatly impact the relative importance of pests. The bird cherry-oat aphid, *Rhopalosiphum padi* L. is probably the major pest of temperate cereal crops on a world scale, it attacks all the major cereals and pasture grasses. The organic sector is in need of alternative aphicides or products that can repel this pest. In spite of the properties of plant volatiles that allow them to act as insect repellents, there is a lack of such products on the market for the agricultural sector. In this work, we tested a group of essential oils and pure compounds in a laboratory choice bioassay with *R. padi* (20 replications per product) and the repellency index (R.I.) was computed after 24 h. At 0.15 μl/cm^2, aniseed, peppermint and lemongrass essential oils were repellent for apterous females. *trans*-Anethole and caryophyllene exhibited volatile toxicity to the insects (LD50 = 0.11 μl/cm^2). R.I. values ranging from 68.8 to 100 were obtained using farnesol, geraniol, *cis*-jasmone, citral, linalool, estragole, pulegone and caryophyllene. Water emulsions of the active products were obtained (nanoemulsions with oil droplets less than 100 nm via ultrasounds for 10 min) and applied at increasing volumes using a computer-controlled spraying apparatus for the bioassay, and a dose response was obtained. Some products were active: carvone increased mobility, whilst *cis*-jasmone repelled *R. padi* at a very low dose (0.02 μl/cm^2 of the treated leaf). Zetasizer measurements indicated that the smaller the particle size within the nanoemulsion, the higher the activity. Using lecithin (1:2) or lecithin plus glycerol (1:2:1) in addition to a bioactive produced larger negative Z-potential values and therefore more stable formulations without any evident effect on activity.

1. Introduction

Some essential oils (EOs), including lemon, peppermint and citronella, are produced worldwide at over 100 t/year. Other EOs, such as basil, are in the 50–100 t/year production range (or even less). Common prices are approximately 6–45 €/Kg of oil. For plant protection purposes, the main commercial EOs are those that contain eugenol (e.g., clove and bay oils); however, pine, anise, eucalyptus and thyme are also used (Lubbe and Verpoorte, 2011). Plant materials cultivated for a specific compound or group of compounds should be standardized, i.e., cultivated in such a way that the level of the desired compound is known and a sufficient amount is available at a constant supply.

The most effective insect repellents are synthetic DEET (diethyl toluamide) and natural citronella oil (Mumcuoglu et al., 1996), which are common ingredients in mosquito repellent sprays. PMD (*p*-menthane-3,8-diol), isolated from mint, is also a common active ingredient (González-Coloma et al., 2010).

A list of plant species with insect repellent properties and their active products can be found in the literature (Isman and Machal, 2006; Koul et al., 2008; Khallaayoune et al., 2009; González-Coloma et al., 2010; Regnault-Roger, 2013) and includes *Artemisia vulgaris* (thuyone, cineole), *Cinnamomum camphora* (cinnamaldehyde), *Curcuma longa* (turmerone), *Eucalyptus* sp, *Myrtus communis* and *Rosmarinus officinalis* (cineole), *Juniperus virginiana* (α and β pinene, methyl-eugenol), *Lavandula angustifolia* (linalool, lynalyl acetate), *Litsea cubeta* and *Cymbopogon* species (citral, citronellal, citronellol), *Melaleuca leucadendron* (terpineol, γ-terpinene), *Mentha pulegium* (pulegone), *Mentha piperita* (menthone, menthol), *Nepeta cataria* (nepetalactone), *Pelargonium* sp. (geraniol), *Syzygium aromaticum* (eugenol), *Thymus* sp. and *Origanum vulgare* (thymol, carvacrol, *p*-cymene).

The repellent properties and fumigant activities of EOs and extracts from species in the genus *Mentha* against mosquitoes, cockroaches and stored product pests are well-documented (Ngoh et al. 1998; Kumar et al., 2011). Peppermint (*Mentha piperita* L.) is an effective repellent

* Corresponding author.
E-mail address: mjesus.pascual@carm.es (M.J. Pascual-Villalobos).

http://dx.doi.org/10.1016/j.indcrop.2017.05.019
Received 6 March 2017; Received in revised form 9 May 2017; Accepted 12 May 2017

Table 1
Products tested.

Product	Type	Source
Plant essential oils		
Aniseed (*Pimpinella anisum* L.)	Essential oil	Destilerías Muñoz Galvez S.A., Murcia
Basil (*Ocimum basilicum* L.)	Essential oil	Destilerías Muñoz Galvez S.A., Murcia
Coriander (*Coriandrum sativum* L.)	Essential oil	Destilerías Muñoz Gálvez S.A., Murcia
Fennel (*Foeniculum vulgare* Miller)	Essential oil	Destilerías Muñoz Gálvez S.A., Murcia
Lemon (*Citrus limon* (L.) Burm. f), cold extraction of organic fruits	Essential oil	Citromil S.L., Santomera, Murcia
Lemon (*Citrus limon* (L.) Burm. f), distillation of fruits	Essential oil	Citromil S.L., Santomera, Murcia
Lemongrass (*Cymbopogon flexuosus* (Nees ex Steud.) W. Watson	Essential oil	Plants grown in the open field in IMIDA Exp, Stat., Murcia
Peppermint (*Mentha piperita* L.)	Essential oil	Destilerías Muñoz Gálvez S.A., Murcia
Pennyroyal (*Mentha pulegium* L.)	Essential oil	Destilerías Muñoz Gálvez S.A., Murcia
Pine (*Pinus sibirica* Du Tour)	Essential oil	Destilerías Muñoz Gálvez S.A., Murcia
Compounds		
trans-Anethole 99%	Phenylpropanoid	Across Organics
D-Carvone	Monoterpenic Ketone	Sigma Aldrich
β-Caryophyllene	Terpenic Hydrocarbon	Across Organics
Citral 95% (geranial and neral mixture)	Monoterpenic Aldehyde	Aldrich
Estragole (4-allylanisole)	Phenylpropanoid	Sigma Aldrich
Geraniol	Monoterpenic Alcohol	Across Organics
Farnesol	Acyclic Sesquiterpenic Alcohol	Sigma Aldrich
(−)-Fenchone 98%	Monoterpenic Ketone	Alfa Aesar
cis-Hexenol	Leaf Alcohol	Sigma Aldrich
cis-Jasmone	Volatile organic compound	Sigma Aldrich
(R)-(+)-Limonene	Monoterpenic Hydrocarbon	Sigma
Linalool	Monoterpenic Alcohol	Sigma Aldrich
Menthone (mixture of isomers)	Monoterpenic Ketone	Alfa Aesar
DL-Menthol 98%	Monoterpenic Alcohol	Alfa Aesar
β-Pinene	Monoterpenic Hydrocarbon	Across Organics
(R)-(+)-Pulegone	Monoterpenic Ketone	Sigma Aldrich
Methyl salicilate 98%	Organic Ester	Alfa Aesar
γ-Terpinene 98%	Monoterpenic Hydrocarbon	Across Organics

and pennyroyal (*Mentha pulegium* L.) is an effective fumigant against flies and red mites. However, improvements relating to the storability, persistence and efficacy of such products have yet to be made.

In a choice bioassay used to test the oviposition inhibition of *Callosobruchus maculatus* F. (Coleoptera: Bruchidae), basil EOs rich in estragole, linalool, geraniol or geranial exhibited repellent effects; only those with higher contents of estragole were toxic to insects (Pascual-Villalobos and Ballesta-Acosta, 2003).

Dry or fresh leaves of *Ocimum kenyense* Ayob. ex A.J. Paton and *Ocimum kilimadscharicum* Baker ex Gürke, or their EOs, available as local materials in East Africa, are used to protect stored cereals because of their repellent properties against *Sitophilus zeamais* (Jembere et al., 1995; Bekele et al., 1997).

According to a review by Pavela (2015), plant species with the ability to be cultivated for the production of EOs as mosquito repellents in Europe include the Apiaceae: *Pimpinella anisum* L., *Coriandrum sativum* L. and *Foeniculum vulgare* Miller. Lemongrass (*Cymbopogon flexuosus*) is a perennial aromatic grass that is mainly produced in India, with its EO used in soaps, insect repellents and cosmetics.

The fumigant toxicity of EOs of cumin, anise, origanum and eucalyptus against the cotton aphid (*Aphis gossypii*) has been reported (Isman, 2000). Other natural products cited for their effects on aphids are vetiver oil and derivatives from orange and lemon fruits or pine trees. Thymol and menthol are effective against Varroa mites.

We previously tested liquid spray formulations of 2.38% *trans*-anethole, carvone and linalool against *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). *trans*-Anethole was shown to be more toxic to insects than carvone or linalool. Surviving females of the latter cases, however, demonstrated reduced fertility (Pascual-Villalobos et al., 2014). In another experiment, we tested encapsulated (solid beads) coriander and basil EOs as killing agents inside of funnel traps to monitor *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae); the EO had a similar performance to that of the conventional vapone (dichlorvos) insecticide (Pascual-Villalobos et al., 2015).

The bird cherry-oat aphid, *Rhopalosiphum padi* L. is one of the 14 aphid species of most agricultural importance. It attacks all the major cereals and pasture grasses, and is probably the major pest of temperate cereal crops on a world scale (Blackman and Eastop, 2007). It is also vector of Barley Yellow Dwarf Virus (BYDV). With climate change and milder winters, increased insect survival and earlier migration are expected to increase the severity of crop damage. Systemic insecticides are effective, but only if sprayed during significant infestations. The organic sector is in need of alternative aphicides or products that prevent pests. In spite of the properties of plant volatiles as insect repellents, there is a lack of such products on the market for the agricultural sector. Repellents could be useful in integrated pest management strategies in the context of the so-called SDDS (stimulo-deterrent diversionary strategy) or pull-push.

Nanopesticides represent an emerging technological development that could offer increased efficacy, durability and reduction in the current amount of active ingredients used. They are formulated from materials in the range of 1–100 nm in at least one dimension. Some authors have already published work related to the formulation of EOs as emulsions (Kumar et al., 2013).

The objective of our work was to test a group of plant EOs and pure compounds on *R. padi* with a laboratory bioassay, to select active products to be formulated as nanoemulsions and spray the preparations, to demonstrate that aphid repellency occurs at increasing doses, and to ultimately characterize the emulsions to identify parameters that would be useful in the optimization of formulates.

2. Material and methods

2.1. Essential oils and pure compounds

Ten plant EOs were studied, 3 Umbelliferae, 3 Labiatae, 2 Rutaceae, 1 Graminaceae and 1 Pinaceae (Table 1), together with a group of 18 pure compounds that included phenylpropanoids (*trans*-anethole and estragole), monoterpenic ketones (carvone, fenchone, menthone and pulegone), monoterpenic aldehydes (citral), monoterpenic (geraniol,