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Use of nanoemulsions of plant essential oils as aphid repellents

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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

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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,

Table 2
Chemical composition of plant essential oils (GC–MS).

Main compounds	R.I.	% Area by GC
Aniseed (*Pimpinella anisum* L.)		
trans-Anethole	1514	96.9
Total identified	–	99.6
Basil (*Ocimum basilicum* L.)		
Estragole	1317	79.2
Linalool	1145	15.5
Total identified	–	99,7
Coriander (*Coriandrum sativum* L.)		
Linalool	1145	69.9
Camphor	1215	6.2
Geranyl acetate	1727	4.9
α-Pinene	946	4.8
Total identified	–	99.6
Fennel (*Foeniculum vulgare* Miller)		
trans-Anethole	1514	30.9
Limonene	1042	19.2
α-Phellandrene	1012	14.8
Fenchone	1126	13.1
α-Pinene	946	4.4
Estragole	1317	4.3
Total identified	–	98.9
Lemon (*Citrus limon* (L.) Burm. f), cold extraction of organic fruits		
Limonene	1042	67.6
β-Pinene	984	11.4
γ-Terpinene	1082	8.3
α-Pinene + α-Thuyene	946	2.5
Geraniol	1439	2.2
Geranyl acetate	1727	1.3
Neral	1412	1.1
Total identified	–	98.6
Lemon (*Citrus limon* (L.) Burm. f), distillation of fruits		
Limonene	1042	71.1
β-Pinene	984	11.5
γ-Terpinene	1082	8.2
α-Pinene + α-Thuyene	946	2.1
Geraniol	1439	0.7
Geranyl acetate	1727	0.5
Neral	1412	0.4
Total identified	–	98.9
Lemongrass (*Cymbopogon flexuosus* (Nees ex Steud) W. Watson)		
Citral (geranial + neral)	1480 − 1412	79.7
α-Phellandren-8-ol	1258	4.0
β- Phellandren-8-ol	1304	3.8
Total identified	–	94.6
Peppermint (*Mentha piperita* L.)		
Menthol	1278	40.4
Menthone	1238	23.5
Menthol acetate	1476	8.3
Isomenthone	1251	6.1
Eucalyptol	1045	4.4
Total identified	–	98.6
Pennyroyal (*Mentha pulegium* L.)		
Pulegone	1410	82.4
Menthone	1238	4.4
Total identified	–	94.1
Pine (*Pinus sibirica* Du Tour)		
Borneol acetate	1502	34.5
Camphene	958	18.5
α-Pinene	946	13.9
Δ-3-Carene	1048	10.9
Limonene	1082	8.9
Total identified	–	98.4

Individual peaks were identified by retention times and retention indices (R.I.) on HP-5 capillary column (relative to C9–C20 *n*-alkanes), compared with those of known compounds, and identified by comparison of mass spectra using the NBS75K library and spectra obtained from the standard.

linalool and menthol) and other (farnesol and *cis*-hexenol) alcohols, monoterpenic hydrocarbons (limonene and γ-terpinene) and other products (caryophyllene, *cis*-jasmone and methyl salicilate).

EOs were subjected to analysis by gas chromatography–mass spectrometry (GC–MS). An Agilent, model 6890 N, GC (Agilent Technologies, Palo Alto, CA, USA), equipped with a 30 m × 0.25 μm i.d. HP-5 (5% cross-linked phenyl-methyl siloxane) column with 0.25 μm film thickness was used. Helium was used as the carrier gas (constant pressure, β-ionone eluting at 27.60 min) and the split ratio was set to 100:1 with 0.1 μl of injected sample.The column, was initially at 60 °C, then increased to 155 °C at a rate of 2.5 °C/min, and finally raised to 250 °C at a rate of 10 °C/min. The injection port and the transfer line to the mass selective detector were kept at 250 and 280 °C respectively. The mass spectrometer was operated in electron impact ionization mode with an ionizing energy of 70 eV, scanning from *m/z* 50–350 at 3.21 scan/s. The quadrupole temperature was 150 °C and the electron multiplier voltage was maintained at 1300 V. The individual peaks were identified by the retention times and retention indices (relative to C6–C17 *n*-alkanes), compared with those of known compounds, and by comparison of mass spectra using the NBS75K library and spectra obtained from standards. Percentage compositions of samples were calculated according to the area of the chromatographic peaks using the total ion current. The main compounds identified for each oil are summarized in Table 2, although other compounds were also present and chemically identified.

Table 3
Repellent activity of essential oils (0.15 μl/cm^2) against *Rhopalosiphum padi* L. in choice bioassays after 24 h.

		Repellency			Mortality
	Assay[a]	R.I.[b]	RD50[c]	RD90[c]	%
Aniseed	C	84.3 ± 6.58	–	–	30.5
	V	58.4 ± 8.60	0.14 (0.13–0.15)	0.17 (0.16–0.18)	13.0
Basil	C	47.2 ± 10.3	–	–	14.0
	V	42.4 ± 8.32	–	–	7.0
Coriander	C	28.2 ± 8.49	–	–	33.9
	V	30.0 ± 8.73	–	–	36.0
Fennel	C	11.3 ± 4.18	–	–	7.5
	V	11.0 ± 4.71	–	–	10.0
Lemon cold extraction	C	22.4 ± 7.18	–	–	22.0
	V	18.0 ± 6.42	–	–	20.5
Lemon distillation	C	14.3 ± 6.68	–	–	41.2
	V	39.2 ± 7.51	–	–	36.0
Lemongrass	C	63.4 ± 6.64	0.12 (0.11–0.12)	0.15 (0.14–0.15)	7.5
	V	66.7 ± 7.20	0.08 (0.07–0.09)	0.12 (0.11–0.13)	5.0
Peppermint	C	72.0 ± 6.29	0.13 (0.13–0.14)	0.16 (0.16–0.17)	16.5
	V	44.8 ± 7.62	0.12 (0.11–0.13)	0.20 (0.18–0.22)	13.5
Pennyroyal	C	15.4 ± 5.65	–	–	6.5
	V	27.9 ± 6.91	–	–	2.5
Pine	C	17.9 ± 5.55	–	–	15.0
	V	12.3 ± 5.43	–	–	15.0

[a] C = air tight (lid of plastic box without opening), V = ventilated (lid of plastic box with a 1 cm^2 opening covered with a mesh); The bioassay consisted of 20 replications in a 2.2 × 2.2 × 1 cm^3 plastic box with treated and control barley leaves (on agar) offered to 10 apterous females and maintained at 22 °C and 16:8 h photoperiod. The essential oils were applied as acetone solutions.

[b] Repellency Index (R.I.) = [1 − (T/C)] × 100 where, T = number of aphids on treated leaf after 24 h and C = number of aphids on control leaf after 24 h. Replications with less than 40% aphid settlement were omitted for computation. Activity was considered if R.I. ≥ 75.

[c] RD50 and RD90 are the doses of essential oils (μl/cm^2) that give R.I. values of 50 and 90, respectively. Doses were calculated in active essential oils only. 95% Confidence Intervals have been calculated according to Trevors and Lusty (1985). C (air tight) assay resulted in high aphid mortality with aniseed, and therefore, the doses could not be calculated.

Table 4
Repellent activity of compounds (0.15 μl/cm^2) against *Rhopalosiphum padi* L. in choice bioassays after 24 h.

		Repellency			Mortality
	Assay[a]	R.I.[b]	RD50[c]	RD90[c]	%
trans-Anethole	C	–	–	–	59.0
	V	–	0.13 (0.12–0.13)	0.16 (0.16–0.17)	56.5
Carvone	C	48.4 ± 9.01	0.12 (0.11–0.14)	0.19 (0.18–0.20)	12.0
	V	61.5 ± 10.9	0.16 (0.15–0.17)	0.19 (0.19–0.20)	38.5
Caryophyllene	C	–	–	–	53.5
	V	80.5 ± 4.49	–	–	20.0
Citral	C	78.0 ± 5.96	0.08 (0.007–0.08)	0.11 (0.1–0.11)	27.5
	V	79.2 ± 6.93	0.05 (0.05–0.05)	0.07 (0.07–0.07)	24.2
Estragole	C	68.8 ± 7.87	0.13 (0.12–0.13)	0.15 (0.14–0.16)	10.5
	V	76.2 ± 7.32	0.1 (0.1–0.11)	0.13 (0.12–0.13)	10.5
Geraniol	C	99.3 ± 6.71	0.05 (0.05–0.05)	0.07 (0.06–0.07)	24.0
	V	96.2 ± 1.79	0.05 (0.05–0.05)	0.07 (0.06–0.07)	11.5
Farnesol	C	98.7 ± 0.88	0.03 (0.03–0.03)	0.04 (0.04–0.05)	32.5
	V	100 ± 0.00	0.03 (0.03–0.04)	0.05 (0.04–0.05)	31.0
Fenchone	C	27.5 ± 7.61	–	–	10.5
	V	32.8 ± 7.77	–	–	7.0
cis-Hexenol	C	62.8 ± 7.89	0.27 (0.21–0.35)	0.52 (0.33–0.84)	6.5
	V	80.0 ± 4.28	–	–	5.0
cis-Jasmone	C	85.8 ± 3.74	0.05 (0.05–0.05)	0.07 (0.07–0.08)	39.5
	V	82.6 ± 4.42	0.05 (0.05–0.06)	0.08 (0.08–0.09)	22.0
Limonene	C	20.9 ± 7.32	–	–	27.5
	V	33.1 ± 7.63	–	–	21.5
Linalool	C	85.2 ± 5.90	0.09 (0.08–0.09)	0.11 (0.10–0.11)	17.5
	V	90.7 ± 3.73	0.09 (0.09–0.10)	0.12 (0.12–0.12)	18.5
Menthone	C	–	0.15 (0.13–0.16)	0.23 (0.20–0.27)	89.5
	V	–	0.15 (0.13–0.16)	0.25 (0.20–0.31)	46.5
Menthol	C	–	0.04 (0.03–0.04)	0.08 (0.07–0.08)	62.5
	V	–	0.06 (0.05–0.08)	0.19 (0.14–0.25)	49.0
β-Pinene	C	19.3 ± 4.73	–	–	4.5
	V	24.1 ± 6.32	–	–	4.0
Pulegone	C	–	0.13 (0.12–0.14)	0.20 (0.17–0.23)	68.5
	V	74.5 ± 8.89	0.13 (0.12–0.14)	0.26 (0.22–0.3)	31.5
Methyl salicilate	C	15.1 ± 5.41	–	–	15.5
	V	43.2 ± 7.14	–	–	7.5
γ-Terpinene	C	17.5 ± 4.82	–	–	36.5
	V	28.2 ± 6.84	–	–	27.0

[a] C = air tight (lid of plastic box without opening), V = ventilated (lid of plastic box with a 1 cm^2 opening covered with a mesh); The bioassay consisted of 20 replications in a 2.2 × 2.2 × 1 cm^3 plastic box with treated and control barley leaves (on agar) offered to 10 apterous females and maintained at 22 °C and 16:8 h photoperiod. The compounds were applied as acetone solutions.

[b] Repellency Index (R.I.) = [1 − (T/C)] × 100 where, T = number of aphids on treated leaf after 24 h and C = number of aphids on control leaf after 24 h. Replications with less than 40% aphid settlement were omitted for computation. Activity was considered if R.I. ≥ 75.

[c] RD50 and RD90 are the doses of compounds (μl/cm^2) that give R.I. values of 50 and 90, respectively. Doses were calculated in active products only. 95% Confidence Intervals have been calculated according to Trevors and Lusty (1985).

2.2. Aphids

R. padi L. (Homoptera: Aphididae) was reared on banker barley plants (13 × 13 × 13 cm^3 pots were sown with 40 ml of barley seed to yield a very high plant density) under controlled conditions at a constant temperature of 24 °C and a 16:8 h photoperiod. Third instar nymphs were used for the bioassay.

Table 5
Toxicity of products against *Rhopalosiphum padi* L. in choice bioassays[a] after 24 h.

	LD50[b]	LD90[b]
Aniseed	0.14 (0.14–0.15)	0.18 (0.18–0.19)
trans-Anethole	0.11 (0.11–0.11)	0.14 (0.14–0.15)
Caryophyllene	0.12 (0.12–0.12)	0.14 (0.14–0.14)
Estragole	0.22 (0.22–0.23)	0.25 (0.24–0.25
Pulegone	0.21 (0.20–0.23)	0.41 (0.37–0.46)

[a] C = air tight (lid of plastic box without opening); The bioassay consisted of 20 replications in a 2.2 × 2.2 × 1 cm^3 plastic box with treated and control barley leaves (on agar) offered to 10 apterous females and maintained at 22 °C and 16:8 h photoperiod. The compounds were applied as acetone solutions.

[b] LD50 and LD90 are the doses of compounds (µl/cm^2) that cause 50% or 90% mortality, respectively. Lethal doses were calculated by probit analysis in products that exhibited insecticidal activity (≥30% mortality) at 0.15 µl/cm^2 (see Tables 3 and 4) and gave a response to the dose in the bioassay. 95% Confidence Intervals have been calculated according to Trevors and Lusty (1985).

2.3. Bioassay and initial screening of products

The methodology described in this Section applies for Tables 3–5 .

The choice bioassay used was described by Gutiérrez et al. (1997). The EOs and pure compounds were applied to barley leaves with a pipette (10 µl/cm^2) as acetone solutions prepared at 1.5%, which was equivalent to 0.15 µl of the bioactive per cm^2 for the initial screening. Control leaves were treated with solutions prepared in the same manner, but without the bioactive. The repellency index (R.I.) was calculated after 24 h.

For all products, bioassays were performed with two types of boxes, one in which the lid had an opening (1 cm diam.) covered with mesh (V = ventilated bioassay) and another in which the lid had no opening (C = air tight bioassay) to enhance the volatile action. Control leaves were treated with solutions prepared in the same manner, but without the bioactive.

For the active products RD50 and RD90, doses of EOs or compounds (µl/cm^2) that gave R.I. values of 50 and 90, respectively, were investigated. Mortality (%) was also recorded after 24 h, and LD50 and LD90, the doses of EOs or compounds (µl/cm^2) that cause 50% or 90% mortality, respectively, were investigated by probit analysis in products that exhibited insecticidal activity (≥30% mortality). 95% Confidence Intervals were calculated according to Trevors and Lusty (1985).

2.4. Nanoemulsions of active products

2.4.1. Preparation and formulation

Water emulsions of active products (at 5%) were prepared with Tween 80 (1:1). Ultrasounds (output = 5 and pulse = 40%) was applied with a standard horn of a 13 mm tip diam. for 10 min using a Vibra-Cell 50/60 Hz, 375-W High Intensity Ultrasonic Liquid Processor Vibracell (Sonics & Materials, Inc., Connecticut, USA). The sonicator probe was submerged to the center of the processed volume (200 ml). Control nanoemulsions were prepared in the same manner, but in the absence of the bioactive. All nanoemulsions were sprayed afterwards using a computer-controlled spraying apparatus (CSSA), as described in the following section.

Under the same conditions of the ultrasounds (except with the pulser off and 100 ml volumes being processed), 1% nanoemulsions were prepared with the active products: Tween 80 (1:2), soy lecithin (1:2), and soy lecithin plus glycerol (1:2:1). Control nanoemulsions were prepared in the same manner, but in the absence of the bioactive. All nanoemulsions were sprayed afterwards using a CSSA, as described in the following section. The nanoemulsions were used for characterization as described in Section 2.4.3.

2.4.2. Application using a computer controlled spraying apparatus (CCSA)

A CCSA (Burkard Manufacturing Co. Ltd., England) was used to treat barley leaf pieces for the bioassay. The sprayer was calibrated to deliver 0.02, 0.29 and 0.5 µl of the bioactive per cm^2 feeding on the 5% nanoemulsions prepared. The bioassay was performed as described in Section 2.3. In addition to determining the R.I. for each dose, aphid settlement (% on the leaves) and mortality (%) were also recorded after 24 h. The results are graphically represented in Fig. 1 and in Supplementary file – Annex I.

The CCSA and same bioassay were used with the prepared 1% nanoemulsions; however, only one dose (0.06 or 0.12 µl of the bioactive per cm^2) was applied for the series of 3 formulations prepared. The R.I. was calculated after 24 h. Transformed data [arcsin sqrt (R.I./100)] were analysed by ANOVA considering product and formulation as factors. The results are presented in Figs. 2,4,6–8 as well as in the Supplementary file – Annex II.

2.4.3. Characterization

A Zetasizer Nano ZS (Malvern Instruments Ltd., UK) was used to measure the particle size in the nanoemulsions by dynamic light scattering (DLS). Three measurements were taken per sample. The

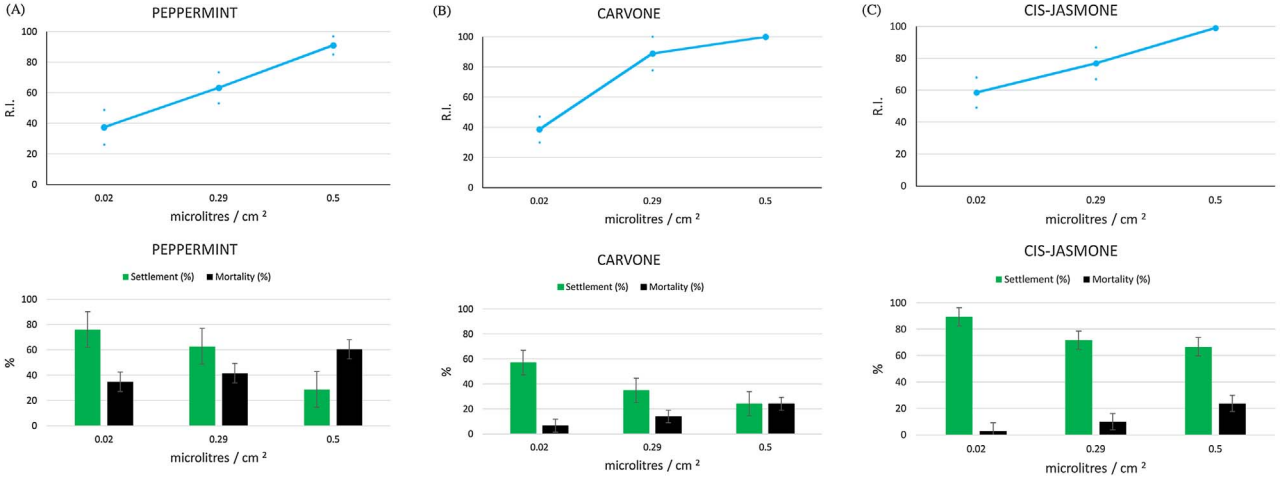
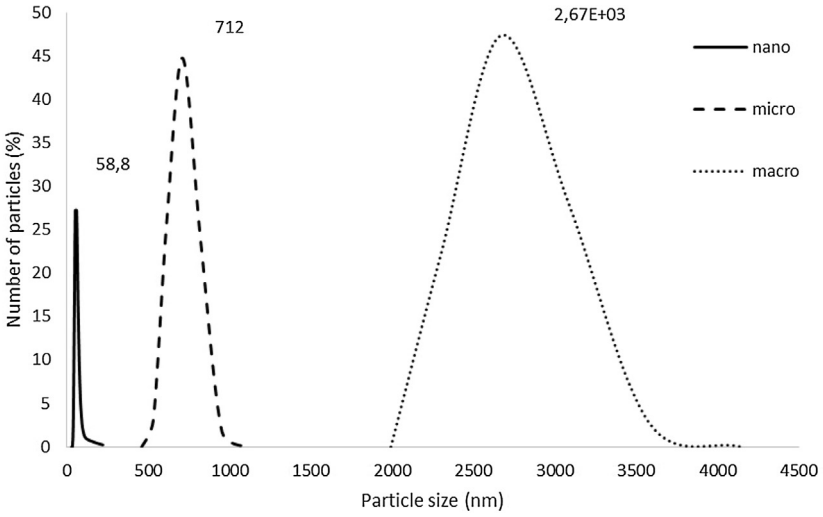


Fig. 1. Repellency Index (R.I.) after 24 h of (A) peppermint essential oil, (B) carvone or (C) *cis*-Jasmone sprayed (as water-based nanoemulsions) onto barley leaves at increasing doses in choice ventilated bioassays of *R. padi*.



	D(4,3)	D 0.9	D 0.5	PDI	Z-Potential	Activity (R.I)
nano	99	78.8	58.8	0.36	-17.9	66.3 ± 7.53
micro	816	825	712	0.713	-9.95	25.8 ± 6.34
macro	3136	2500	2670	0.615	-13.8	34.5 ± 7.96

D(4,3) = average volumen distribution diameter (nm). D 0.9 and D 0.5 = 90 % and 50 % quantiles of the volumen distribution (nm). PDI = polydispersity index.

Fig. 2. Emulsions of 2% citral with a different particle size and repellency index (R.I.) at 0.12 µl of citral/cm^2 against *R. padi.*

results are presented as particle size distribution curves with an average volume distribution diameter, 50% and 90% percentile distributions, polydispersity index and Z-potential (see Figs. 2,4,6 and 9 and Supplementary file – Annex II).

The number of particles/ml or concentration (Supplementary files – Annex II) was counted using a NanoSight with blue laser 488 nm (Malvern Instruments Ltd., UK) for the series of farnesol and citral 1% nanoemulsions prepared as described in Section 2.4.1 The movement of particles in the fluid was visualized and recorded (Supplementary files – Annex III) in 3 videos of 60 s each.

3. Results and discussion

3.1. Analysis of plant essential oils

The results of the GC–MS analyses are summarized in Table 2. Four of the EOs were rich in one main compound: aniseed with 96.9% *trans*-anethole, coriander with 69.9% linalool, lemongrass with 79.7% citral and pennyroyal with 82.4% pulegone. In some cases, two main compounds were identified, in basil (estragole and linalool) or peppermint (menthol and menthone) oils. In addition to limonene (67.6–71.1%), which was abundant in lemon oils, 2–3 additional compounds were present at significant amounts; the same was true for coriander. The fennel EO used for the experiment contained a mixture of four main compounds: *trans*-anethole (30.9%), limonene (19.2%), α-phellandrene (14.8%) and fenchone (13.1%).

3.2. Activity of EOs and pure compounds against R. padi (choice bioassays: air tight and ventilated)

The results of the bioassays are presented in Tables 3–5. In the initial screening, the products were applied in an acetone solution to the barley leaves and left to dry. The R.I. ranged from 11 to 84.3 in EOs and from 15.1 to 100 in pure compounds.

The oils that were more repellent to *R.* padi were aniseed, peppermint and lemongrass. In the first two cases, the effect was more evident (R.I. = 84.3 versus R.I. = 58.4 or R.I. = 72 versus R.I. = 44.8) if there was no opening (covered with mesh) in the lid of the plastic boxes (C bioassay), indicating a volatile action of the product on insect behaviour. By contrast (see Table 3), lemongrass oil was equally repellent (R.I. = 63.4 or 66.7) regardless of whether the lid had an opening or not (C and V bioassays). Some insect mortality (> 30%) was observed when coriander, lemon from distillation or aniseed oils were used.

Table 4 shows that some compounds (*trans*-anethole, menthone and menthol) were toxic to *R. padi* (46.5–89.5% mortality), and therefore, the R.I. could not be computed initally. However, a clear repellent effect (R.I. from 68.8 to 100) was obtained in farnesol, geraniol, *cis*-jasmone, linalool, citral and estragole, with similar R.I. values in both bioassay types (C and V) at a dose of 0.15 µl/cm^2. Additionally, pulegone and caryphyllene were repellent (R.I. = 74.5 and 80.5, respectively) to *R. padi* in the ventilated bioassay, but the vapour concentration was toxic in the air tight bioassay. Pennyroyal was less active than peppermint (Table 3), but their oils have a different composition (Table 2). Pulegone, the main compound (82.4%) of pennyroyal, was more active when applied as a pure compound than as an EO (Tables 3 and 4). An antagonistic effect of menthone was observed against *Drosophila melanogaster* when mixed with pulegone (Franzios et al., 1997).

The dose required to produce a R.I. = 50 (see Tables 3 and 4) was higher in the case of EOs (RD50 = 0.08–0.14 µl/cm^2) compared to pure compounds (RD50 = 0.03–0.15 µl/cm^2). It was interesting that lemongrass had lower RD90 in the ventilated assay than in the air tight one (0.12 and 0.15 µl/cm^2 respectively) indicating some persistence of this oil on the leaf. The most active products were farnesol, geraniol, *cis* jasmone and menthol with RD90 = 0.04, 0.07 or 0.08 µl/cm^2. *cis*-Hexenol was repellent to the aphids but needed greater doses, RD50 = 0.27 µl/cm^2 in the air-tight bioassay and did not respond to

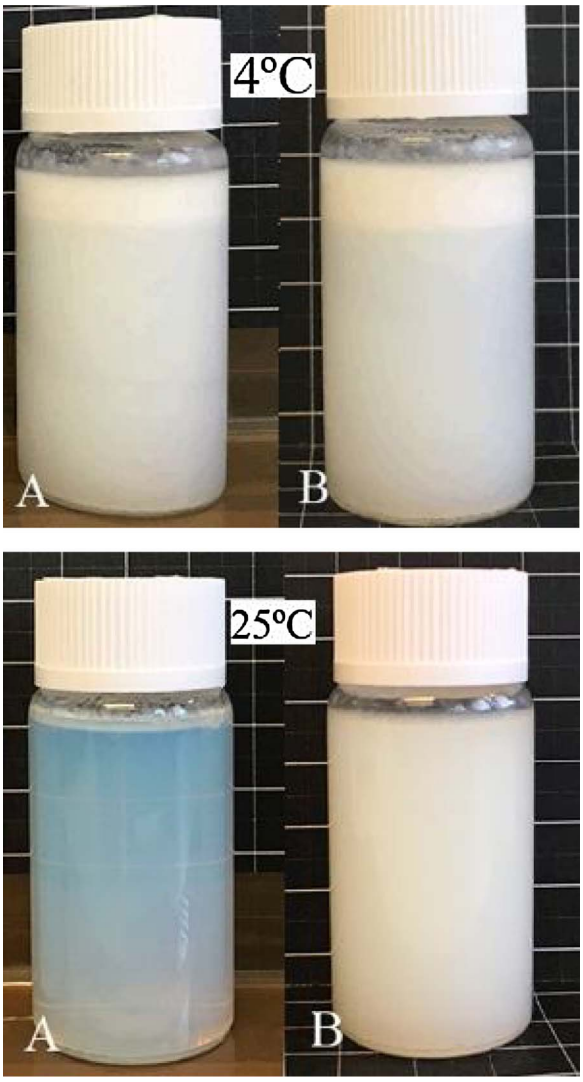


Fig. 3. Stability of a 1% citral nanoemulsion (and Tween 80 1:1) at 4 °C or 25 °C. A: after 15 days of storage. B: after 30 days of storage.

the dose in the ventilated bioassay (Table 4).

Aniseed essential oil and its main compound *trans*-anethole were insecticidal to *R. padi* with LD90 = 0.18 and 0.14 µl/cm^2 respectively (Table 5) and therefore are the most promising for practical application. In addition, a good combination of toxic (C bioassay) and repellent (V bioassay) effects on the aphids is produced, see Tables 3–5: LD90 = 0.14 µl/cm^2 and RD90 = 0.16 µl/cm^2 for *trans*-anethole; LD90 = 0.18 µl/cm^2 and RD90 = 0.17 µl/cm^2 for aniseed. Besides, other products such as caryophyllene, estragole and pulegone were toxic too, with LD90 = 0.14 µl/cm^2, LD90 = 0.25 µl/cm^{2}and LD90 = 0.41 µl/cm^2 respectively for the air-tight bioassay.

As regards the type of bioassay to be used when applying products as acetone solutions, if repellent effects are the main objective of the research then a ventilated assay (V) is more appropriate. Pooling data in Table 3 together and analyzing with arcsin [sqrt (R.I./100)] as dependent variable and assay as factor, yields a non a significant difference (F-ratio = 0.23; d.f. = 1,9; P-value = 0,6464) between both types of bioassays (C and V) for the EOs. As for the pure compounds (data from Table 4), the ventilated assay was more favorable in terms of giving higher values of R.I. (F-ratio = 9.27; d.f. = 1,12; P-value = 0,0102).

On the other hand if mortality is the main objective (and products are applied as acetone solutions) then an air-tight assay is more convenient since there was a significant difference (F-ratio = 4.87; d.f. = 1,9; P-value = 0,05) when arcsin [sqrt (Mortality)] was the input in the ANOVA (EOs data from Table 3). The same was true with data of the pure compounds (from Table 4), giving F-ratio = 6.19; d.f. = 1,17; P-value = 0,0235. Therefore we can conclude that volatile toxicity is enhanced in this type of assay.

3.3. Nanoemulsions

3.3.1. Effect of active products against R. padi (choice bioassay: ventilated)

Applications were performed by spraying nanoemulsions. A graphical representation of the response of *R. padi* to spraying increasing doses of nanoemulsions (from all active products) is included in Supplementary file – Annex I (only the ventilated bioassay is shown). For each product, R.I. values for 3 doses are drawn as a line graph and corresponding histograms for the average mortality and settlement percentages.

In Fig. 1, three of those products are shown to describe the more relevant results. Aphids had a good response to the dose after spraying peppermint. The R.I. increased from 40 to 90 as 0.5 µl/cm^2 was applied instead of the lower 0.02 µl/cm^2 dose (Fig. 1A). An increase in mortality was also observed and therefore decreased aphid leaf settlement.

Carvone, the monoterpenic ketone caused increased insect mobility. Aphid settlement (Fig. 1B) was low (30–60%); however, insects were alive (see the low mortality figures) and moving within the box but had not settled on the leaves. This behaviour may be of interest for pest control since aphids would be more exposed to chemical treatments or natural enemies and thus spend less time reproducing. Additionally, repellency was observed at doses of 0.29 µl/cm^2 onwards. Increased mobility of aphids has been reported for β-farnesene (the aphid alarm pheromone), which was further enhanced when mixed at 1 g/ha with a pyrethroid (Pickett, 1989). A mixture of eugenol and thymol induced behavioural effects in 2nd instar nymphs of the green peach aphid (*Myzus persicae* Sulzer) in a laboratory bioassay (Isman, 2000); less than 50% of aphids were feeding on leaves and were found walking in the Petri dish or dead.

cis-Jasmone is a volatile organic compound that proved to be quite repellent (R.I. = 58.6) at the lowest dose of 0.02 µl/cm^2 (Fig. 1C). Neither a toxic effect nor a low settlement were observed at any of the tested doses.

As shown in the Supplementary file – Annex I, two compounds, caryophyllene and *cis*-hexenol, were active when applied in acetone solution (Table 4), but did not give good results (R.I. < 50 and R.I. < 30, respectively) when applied as 5% nanoemulsions. Therefore, their miscibility with water should be studied or overcome. *cis*-Hexenol is a volatile emitted by wheat seedlings that are either infested or not infested with *R. padi*; therefore, it is a green plant volatile rather than an insect volatile (Quiroz et al., 1997).

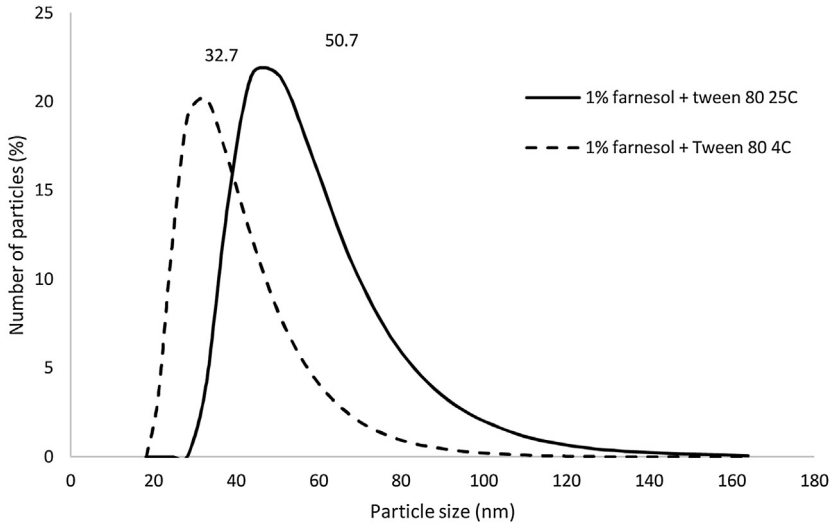
3.3.2. Characterization and activity

Water-based emulsions of products were characterized using a Zetasizer. The volume particle size distribution was measured together with an estimation of the average diameter, 90% and 50% percentiles and polydispersity index (Figs. 2, 4, 6 and Supplementary file – Annex II).

Particle size had an influence on repellency (see Fig. 2), with smaller particles (99 nm of a 2% citral nanoemulsion) producing greater values of R.I. (=66.3) and larger particles (816 or 3136 nm) giving inactive emulsions.

Regarding the stability of the nanoemulsions, the storage temperature was important for citral and farnesol. As shown in Fig. 3, at 4 °C, a 1% citral emulsion separated into two layers after 15 days. The same formulation at 25 °C gave a clear stable nanoemulsion for 15 days, but began to flocculate (giving a milky appearance) after 30 days of storage.

In the case of farnesol, we did not observe visual infestability after 3



	D(4,3)	D 0.9	D 0.5	PDI	Z-Potential	Activity (R.I.)
25ºC	86	68.1	50.7	0.123	-15.9	36.2 ± 9.20
4ºC	74	50.7	32.7	0.132	-12.2	77.3 ± 6.13

D(4,3) = average volumen distribution diameter (nm). D 0.9 and D 0.5 = 90 % and 50 % quantiles of the volumen distribution (nm). PDI = polydispersity index.

Fig. 4. Nanoemulsions of 1% farnesol (and Tween 80 1:1) stored for 3 months at different temperatures. Particle size distribution and repellency index (R.I.) at 0.06 µl of farnesol/cm^2 against *R. padi.*

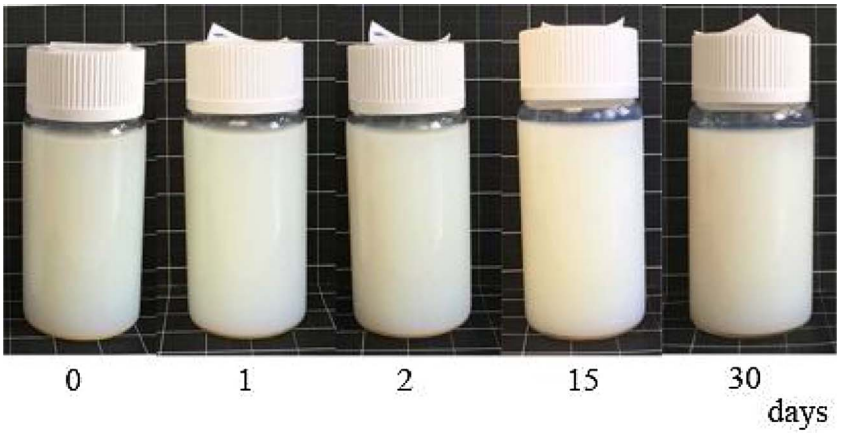


Fig. 5. Stability of a concentrated (10%) nanoemulsion of *trans*-anethole (and Tween 80 1:1) stored at 25 °C.

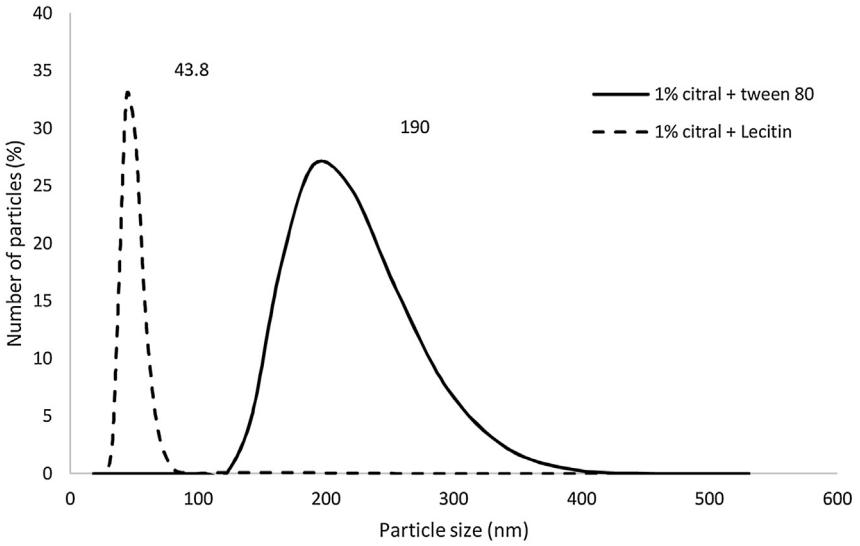
months; however, the resulting samples were repellent (R.I. = 77.3) when stored at 4 °C, but not (R.I. = 36.2) if they were maintained at 25 °C. As shown in Fig. 4, the association between activity and particle size is clear: 90% of droplets in the emulsion were smaller than 68.1 nm at 25 °C and smaller than 50.7 nm at 4 °C.

trans-Anethole appeared to be quite stable when formulated in a diluted or concentrated manner. Fig. 5 visually shows a 10% nanoemulsion during 30 days of storage; no changes were observed, even for long periods of time.

Lecithins can be used as surfactants instead of Tween 80. As shown in Fig. 6, in addition to particle size, Z-potential also gives an indication of emulsion stability (if the values are > +30 or < −30), which could be another factor associated with the aphid repellency of the formulations. A 1% nanoemulsion of citral with lecithin (1:1) produced smaller particles (D[3,4] = 30 nm), a larger negative Z-potential value = −53.9 and an active R.I. value (=93.5), as opposed to an emulsion in Tween 80.

In Supplementary file – Annex II, a series of 3 formulations prepared for each active product is presented in terms of nanoemulsion characteristics and activity. In addition to using lecithin as a surfactant (1:2) in one sample, we included the stabilizer glycerol (1:2:1) in another sample of the series.

With respect to the effects on activity, in some cases, it appeared that using lecithin improved the R.I. value, e.g., aniseed, citral, geraniol, *cis*-jasmone, carvone, estragole, pulegone or caryophyllene. In other cases, R.I. was indifferent, but the R.I. value rarely decreased (with the exception of *cis*-hexenol). In Fig. 7 the histogram for the activity for the series of each product is shown. The ANOVA of arcsin [sqrt (R.I./100)] considering product and formulation as factors gave a significant effect of product (F-ratio = 13.14; d.f. = 13, 26; P-value = < 0.001) but a non significant effect of formulation (F-ratio = 2.42; d.f. = 2, 26; P-value = 0.1079). However, if we exclude *cis*-hexenol from the analysis (on account of being the only nanoemulsion without a dose response in the bioassay, see Supplementary file −



	D(4,3)	D 0.9	D 0.5	PDI	Z-Potential	Activity (R.I.)
Tween 80	324	255	190	0,1	-8.1	30.7 ± 9,38
Lecithin	30	58.8	43.8	0.277	-53.9	93.5 ± 2,95

D(4,3) = average volumen distribution diameter (nm). D 0.9 and D 0.5 = 90 % and 50 % quantiles of the volumen distribution (nm). PDI = polydispersity index.

Fig. 6. Nanoemulsions of 1% citral with different surfactants after 2 months of storage. Particle size distribution and repellency index (R.I.) at 0.12 µl of citral/cm^2 against *R. padi.*

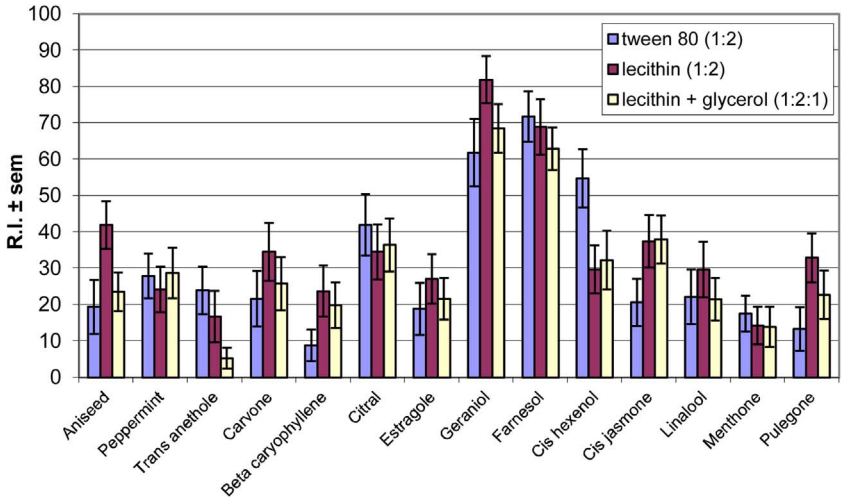


Fig. 7. Activity of the series of three formulations prepared for each product and tested against *R. padi* at 0.06 µl/cm^2 (aniseed, peppermint, geraniol, *cis*-jasmone and farnesol) or at 0.12 µl/cm^2 (all others).

Annex I), a significant effect of formulation was obtained (F-ratio = 4.59; d.f. = 2, 24; P-value = 0.0205). In Fig. 8, the means and LSD intervals for the formulations is shown.

Lucia et al. (2015) reported higher mortality and a more residual effect in a 2% emulsion of pralethrin with soya lecithin as a surfactant. We obtained a higher mortality (25% against 5%) with *trans*-anethole (at 0.12 µl/cm^2) when formulated with lecithin (see Supplementary file –Annex II).

Overall, the Z-potential values were more negative (−60/−30) if lecithin or lecithin plus glycerol were used in the formulation, indicating good stabilizing capacities of both (Fig. 9). Storability was greatly improved, in such formulations too, since less difference between the two Z-potential measurements (after 15 days and 3 months) was obtained in comparison with the formulations with Tween 80. Caryophyllene for instance maintained its Z-potential values and therefore its stability if lecithin was added in the formulation (Fig. 9). On the other hand the series of *cis*-hexenol nanoemulsions always had worst Z-potential values after 3 months regardless the formulation.

Another measurement that is relevant is the number of particles/ml or concentration. The latter was calculated with a NanoSight for the citral and farnesol samples (see Supplementary file – Annex II). Here, the formulation is more concentrated (×10 to 10000 more) if lecithin was used, resulting in better homogenization.

In conclusion, using lecithin (1:2) as a surfactant in the preparation of a nanoemulsion yielded smaller particle sizes and larger negative Z-potential values; therefore, more stable emulsions maintain or improve their activity during storage. If glycerol (1:2;1) is added to the formulation, Z-potential values < −30 are obtained, indicating an

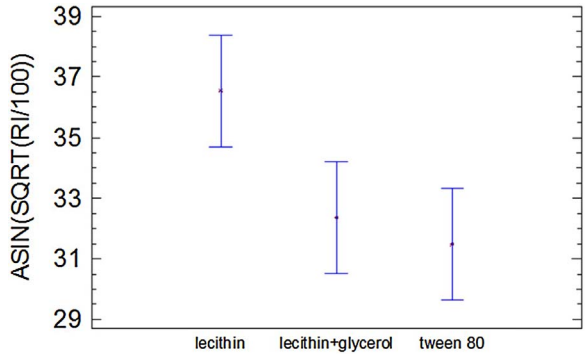


Fig. 8. Means and 95% Fisher's least significant difference (LSD) intervals for the transformed data of the R.I. against *R. padi* for the series of three formulations.

even better stabilizing capacity without any significant changes in activity.

The series of farnesol formulations had R.I. values that ranged from 62.9 to 71.8 at a low dose (0.06 µl of the bioactive/cm^2). A video (obtained with NanoSight) recording the Brownian movement of the particles within the liquid (water) of the 1% farnesol and lecithin (1:2) sample is included in Supplementary file – Annex III.

3.4. General discussion

Lemongrass oil and its main compound citral were repellent to aphids in both bioassays (air tight and ventilated; on average, lemongrass R.I = 65 and citral R.I. = 78.6 at 0.15 µl/cm^2).

Citral is a known insect repellent compound (content of 66–76%) in EOs of tropical grasses of the *Cymbopogon* (lemongrass or citronella) genus (Tajidin et al., 2012). Other sources of citral that are more convenient for temperate and Mediterranean regions (such as crops or byproducts) are *Ocimum citriodorum* Vis, which contain up to 90.7% in its EO (Grayer et al., 1996) or lemon leaf oils (petitgrain), which contain up to 38.7% (Lotta et al., 2002). Lemon peel oils are, in contrast, richer in terpenes, such as limonene, than aromatic compounds. Citral is a flavour component in foods and beverages and is a mixture of geranial and neral (3:2).

The typical aroma of *O. basilicum* is due to estragole (Grayer et al., 1996). This compound is also typical of tarragon (*Artemisia dracunculus* L.). The fennel sample used in our experiments had an intermediate content of *trans*-anethole (a higher amount of this compound and smaller amounts of estragole and fenchone is desirable for plant protection purposes, though). For example, the aniseed sample used herein contained 96.9% *trans*-anethole. The differences in repellency between aniseed and fennel EOs at 0.15 µl/cm^2 against *R. padi* (R.I. = 84.3 and R.I. = 11, respectively) are explained by the greater content of *trans*-anethole in the former (96.9%) than in the latter (30.9%).

Fennel variations in Iranian genotypes accounted for 1.2–88.4% of the *trans*-anethole in the oil (Bahmani et al., 2015), together with estragole (0.22–59.1%), fenchone and limonene. The active compounds can be obtained by distillation or supercritical fluid extraction of seeds; however, the green parts can also be used as raw material, as described by Pavela et al. (2016) for cv. Moravsky that produces a high yield above-ground biomass. Fennel (spice and vegetable) and basil (culinary herb and ornamental) are good candidates for pesticidal plants. We propose that, in Europe, regulations should consider compounds produced from plants that are already used as foods or condiments in terms of low-risk plant protection products to facilitate its commercial development.

In our previous work (Zarrad and Pascual-Villalobos, 2015), *trans*-anethole emulsions (2.38%) exhibited toxic and repellent effects on *R. padi* after treating banker plants (barley) infested with aphids. Previously, Hamraoui and Regnault-Roger (1997) reported that anethole was toxic to *R. padi* after 24 h by inhalation. Kim and Ahn (2001) reported that fennel EO exhibits fumigant activity (penetrating the insect via the respiratory system) on *Sitophilus oryzae* in closed containers (at 0.42 mg/cm^2), but not in open ones. Now our results have demonstrated the aphicidal potential of *trans*-anethole but so far we have not an hypothesis to explain its action into the insect. Among a group of 8 monoterpenoids, *trans*-anethole was the only compound that did not inhibit the enzyme acetylcholinesterase, AChE, in vitro (López and Pascual-Villalobos, 2010).

Gutierrez et al. (1997) previously reported that farnesol and geraniol exhibit repellent effects on aphids, particularly on the green peach aphid (*M. persicae*), with an EC50 = 14.91 µg/cm^2 for farnesol after 1 day and significant mortality at doses of 60 µg/cm^2 after 2 days. Nymphs were more susceptible than adults.

In larger scale experiments (60 × 60 × 60 cm^3 glass unit with windows covered with aphid-proof mesh), farnesol, geraniol, linalool and citronellol repelled alate *R. padi* adults (Halbert et al., 2009) in tiles treated with jelly; the substance was tested at a dose of 0.4 µl/cm^2.

In our experiments clear repellent effects (R.I. from 68.8 to 100) at 0.15 µl/cm^2 were obtained when using farnesol, geraniol, *cis*-jasmone, linalool, estragole, pulegone and caryophyllene.

In regard to the specificity of EOs in aphids, Santana et al. (2012) reported that *Thymus vulgaris* and *Lavandula latifolia* (containing linalool, cineole or linalyl acetate as main compounds) were active on both *R. padi* and *M. persicae*. Additionally, thujone, which contains *Salvia officinalis*, is repellent to the polyphagous *M. persicae*.

Insects detect odours when the volatile binds to odorant receptor proteins that are located on the antennae and maxillary palps and are exposed to the external environment. Regnault-Roger (2013) highlighted the importance of odorant binding proteins (OBPs) and chemosensory proteins (CSPs) in plant-insect interactions: a protein identified in tobacco hornworn (*Manduca sexta*) interacts with plant green volatiles, such as *cis*-hexenol, geraniol and limonene. According to Katz et al. (2008), the future of insect repellents relies on OBPs because they allow air-sprayed versus contact applications. Qiao et al. (2009) reported that, in aphids, OBPs are highly conserved and only a few amino acid changes differ among the proteins found in different species. OBPs are required for odour perception in insects, although other membrane-bound proteins play a role in the correct functioning of the olfactory system in insects. OBPs could become new targets for the development of aphid control agents.

An insect repellent is an odorant that must act in the vapour phase and prevent the insect from coming into contact with plants; otherwise, it would be a tastant or deterrent that acts by contact. Such a vapour barrier is good for products with boiling points close to 110–127 °C. In our experiment, we obtained good repellency for compounds with closer values: farnesol (111 °C), caryophyllene (128 °C), peppermint (130 °C) and *cis*-hexenol (156 °C). Although very good results were obtained in other products (*cis*-jasmone, geraniol or citral) with boiling points above 229 °C, our bioassay may have too small of a unit to draw conclusions. The formulation of an EO should reduce high volatility to keep the active ingredient on the plant surface. According to Nerio et al. (2010), some fixative materials are vanillin, fixed oils or liquid paraffin. From our results, we hypothesize that the use of nanoemulsions might as well keep the vapour barrier more efficiently on the leaf.

Papanikolaou et al. (2017) have published that nano-formulated pyrethrin gave better insecticidal effect compared to commercial products against the aphid *Aphis gossypii* Glover, pointing out to improved dispersion, deposition and leaf adhesion of droplets of the spray solution on leaves surface or easier penetration.

González et al. (2015) formulated geranium and bergamot EO with polyethylene glycol and the nanoparticles produced an increased residual contact toxicity in cockroaches because of the slow and persistent release of the active terpenes. Isman (2000) considers that when insects are exposed to the EO nanoparticles, a decreased detoxifying ratio could occur.

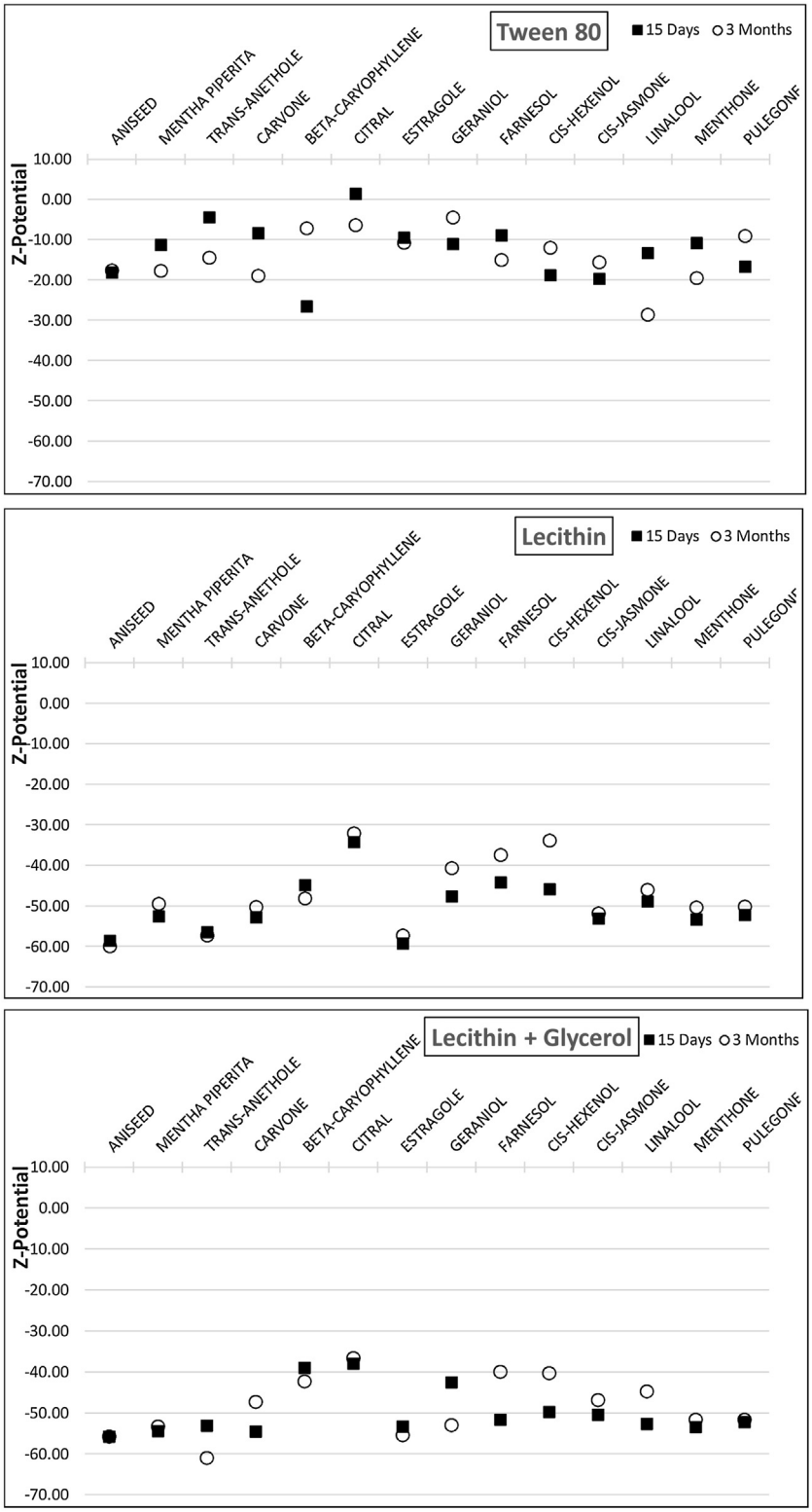


Fig. 9. Z-potential measurements after 15 days or 3 months storage of nanoemulsions of the series of three formulations (Tween 80, lecithin and lecithin plus glycerol) prepared for each product.

Sakulku et al. (2009) reported that the addition of glycerol to a nanoemulsion of citronella oil improved the stability by reducing the droplet size and polydispersity index. Nanoformulations containing up to 15% citronella can be diluted in water without changing the droplet size distribution, as opposed to microformulations.

Moretti et al. (2002) tested EOs formulated as water emulsions on the gypsy moth *Limantria dispar* (Lepidoptera: Lymantridae). The most active EOs (thyme and rosemary) were prepared as controlled release formulations by freeze-drying.

Citral is not stable in water; its degradation occurs under acidic pHs, generating an aqueous phase, an oil phase and an interfacial region (Choi et al., 2009). It is possible to alter the degradation rate by incorporating 0.5% surfactants as microemulsion droplets and 5% triacylglycerol as emulsion droplets; then, citral is trapped inside of

the oil droplets and avoids coming in direct contact with water, making the emulsion more stable. Cationic surfactants better retard the oxidation and good results were obtained by controlling the electrical characteristics of the droplets (interfacial changes) as well (Choi et al., 2010).

Mostafa et al. (2015) reported that fennel EO has antidiabetic efficacy and its formulation with oleic acid enhances its dermal delivery.

A stable emulsion of an EO in water could be obtained if surfactants that have a hydrophilic lipophilic balance (HLB) close to that of the oil phase are used. For example, rosemary oil has an HLB = 15, and Rodríguez-Rojo et al. (2012) reported that *n*-octenil succinic anhydride (OSA-2 and OSA-4) reduced the interfacial tension by two-thirds. Another important factor is the minimum concentration of the surfactant needed to yield a stable emulsion (with the minimum droplet size), which is usually higher than the critical micelle concentration (CMC).

Polymer based formulations in the form of nanogels have been proposed for use in plant protection products with pheromones, EOs or copper as the active ingredients (Shah et al., 2016). The types of polymers considered for nanopesticides consist mainly of polysaccharides (chitosan, alginate and starch), poliesters (poly-ε-caprolactone and polyethylene glycol) or biodegradable materials of biological origin such as gum arabic, beewax, corn oil or lecithins.

Lecithins are complex mixtures of phospholipids that retain oil-soluble substances (such as lavandin oil) in the lipid bilayer membrane. Lecithins have been used by Varona et al. (2013) in formulations of oil in water emulsions that afterwards were processed to solid particles using high-pressure techniques (PGSS and PGSS-drying), as well as spray-drying.

De Oliveira et al. (2014) have published a review on nanotechnology and botanical insecticides. Solid nanoparticles containing carvacrol, thymol or eugenol have been obtained using chitosan, cyclodextrin, nanoclay, polycaprolactone or zein as carrier sytems. Concerning the EOs, the development of nanostructured systems is important for dispersal and to ensure their effective action. For instance, solid lipid nanoparticles reduce the evaporation rate of the EOs compared to reference emulsions (Lai et al., 2006).

In previous works (López et al., 2012) we obtained beads of linalool by an oil-emulsion entrapment method, the encapsulation yield was 86% and the time to 50% release exceded 70 days. On the other hand microcapsules by inverse gelation gave a 69–89% of encapsulation yield and a fast linalool release in one day. The final use of the product will determine which formulations is suitable in each case.

Crop spraying is the most common method used in pest control and the comercial products could be formulated as liquids (soluble concentrates, emulsions etc.) or as solid powders, granules or capsules provided they are water soluble or able to be dispersed in water.

Donsi and Ferrari (2016) consider that nanoemulsions increase the dispersibility of EOs and their stability and therefore their utilization in foods with antimicrobial purposes. Nanoemulsions can be obtained by high pressure homogenization, microfluidization or ultrasounds. According to these authors, the activity of nanoemulsions is broader and stronger than the free EO because droplet size and surface charge influence the transport through cell membranes and the interaction with the multiple molecular sites at the microbial cell membrane. According to Donsi et al. (2012) a sustained release over time of the EOs from the nanoemulsion droplets is produced. EO is partitioned between oil droplets and aqueous phace, acting the oil droplets as nanotanks. The delivery of antimicrobial compounds would occur faster in nanoemulsions due to their reduced particle size and therefore more interfacial area exposed to the microbial cells.

So far the literature on EOs nanoemulsions relate to food science applications. The contribution of our work is that we provide new knowledge on results with insects that are far more complex organisms than microbes. Hopefully more research results on agricultural applications for pest control will be published in the near future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.indcrop.2017.05.019.

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