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ORIGINAL PAPER

Essential oils-based repellents for the management of *Myzus persicae* and *Macrosiphum euphorbiae*

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Abstract

Aphids are one of the major agricultural pests in the world. Their pest management in pepper greenhouses is based on integrated control with release of natural enemies and pesticide treatments used in specific conditions. Essential oils may be used as an eco-friendly alternative for the control of this pest. In this work, we study the repellent effect of essential oils and pure compounds against aphids by two-choice bioassays. *(E)*-anethole, geraniol, farnesol, and *(Z)*-jasmone were the most repellent compounds for *Myzus persicae* and *Macrosiphum euphorbiae* (RD_{50} = 0.011 – 0.086 µl/cm^2). Farnesol at dose of 10 µL attracts natural enemies of aphids, *Aphidius colemani* adults and *Sphaerophoria rueppellii* larvae, in y-tube olfactometer bioassays. A residual toxicity bioassay, using a computer-controlled spraying apparatus, showed a slight toxicity (< 20% mortality) against larvae of *S. rueppellii* when exposed to treatments of *(E)*-anethole, farnesol, or *(Z)*-jasmone individually. The foliar application of farnesol+*(E)*–anethole (1:1) nanoemulsions on plants infested with aphids resulted in a reduction of the population growth ratio of *M. persicae* ($ri = -0.78$) and *M. euphorbiae* ($ri = -3.85$). Among the tested compounds, farnesol is a promising compound to be introduced in aphid management for its potential as a repellent and aphicide, as well as an attractant of some natural enemies of this pest.

Keywords Botanical insecticides · Integrated pest management (IPM) · *Myzus persicae* · *Macrosiphum euphorbiae* · *Sphaerophoria rueppellii* · *Aphidius colemani*

Key message

- Aniseed, basil, and lemongrass essential oils and natural compounds *(E)*-anethole, geraniol, farnesol, and *(Z)*-jasmone are repellent for *Myzus persicae* and *Macrosiphum euphorbiae*.
- Farnesol attracts natural enemies of aphids, *Aphidius colemani* adults and *Sphaerophoria rueppellii* larvae.
- Treatments with farnesol nanoemulsions reduced the aphid population in plants.
- Larvae of *S. rueppellii* are not affected by exposure to the natural compounds tested.
- Farnesol is a promising compound in aphid management and a safe alternative in combination with biological control.

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Introduction

There are more than 5000 species of aphids in the world. Among these, about 450 species have been reported in crop plants. *Myzus persicae* Sulzer (Hemiptera: Aphididae) is one of the most damaging aphids in world agriculture. It is cosmopolitan and polyphagous and is a vector of a high number of plant viruses (Blackman and Eastop 2017). In the 1990s, the control of aphids was mainly performed by organophosphates and carbamates. These compounds are systemic, relatively persistent and highly toxic to aphids but also to non-target insects. In the 2000s, control was mainly based on pyrethroids which are not the most suitable treatments, due to their low persistence and their broad-spectrum action on natural enemies. Neonicotinoids are now widely used (Dewar and Denholm 2017), however, the application of three of the most common neonicotinoids on blooming crops is heavily restricted in Europe (Cressey 2017). Furthermore, *M. persicae* easily generates resistance to pesticides. To date, resistance has been reported to organophosphates, carbamates, pyrethroids, neonicotinoids, and organochlorines (Simon and Peccoud 2018).

In southeastern Spain, sweet pepper is grown in greenhouses using Integrated Pest Management (IPM) strategies combining biological control and synthetic pesticides (Calvo et al. 2012; Dáder et al. 2019). As previously mentioned, the number of authorized insecticides is decreasing due to restrictions or incompatibility with natural enemies and by legal requirements of the European food distribution chains. Biological control of aphids is based on the use of parasitic wasps like *Aphidius colemani* Viereck (Hymenoptera: Braconidae) (Payton Miller and Rebek 2018) or predators like syrphid *Sphaerophoria rueppellii* (Wiedemann) (Diptera: Syrphidae) (Amorós-Jiménez et al. 2015; Calvo-Agudo et al. 2020).

The application of insecticides in the greenhouse may be employed when natural enemies have already settled in the crop. In these cases, pesticides need to be compatible and not harmful to natural enemies. Overcoming these incompatibilities is one of the most complicated aspects in IPM strategies (Rodrigues et al. 2013).

Most essentials oils (EOs) are a complex mixture of monoterpenes, biogenetically related phenols, and sesquiterpenes (Isman 2006). In previous studies, our research group tested the repellent effects of EOs on *Rhopalosiphum padi* (Linnaeus) using a settling inhibition bioassay (Pascual-Villalobos et al. 2017).

Some plants have repellent properties against insects due to Volatile Organic Compounds (VOCs) emitted by them, in fact, these plants can be intercropped in order to protect other harvests from their attack. This is, for example, the case of intercrops of onion, basil, rosemary, or marigolds repelling aphids, thrips, or whiteflies (Hori 1996, 1999a; Dardouri et al. 2019; Guilbaud and Khudr 2020; Ben Issa et al. 2017). Extracts and EOs obtained from these plants often cause repellency/deterrence as well. Hori and Komatsu (1997) reported the repellent effect of rosemary essential oil (EO) and some of its main compounds against the aphid *Neotoxoptera formosana* (Takahashi). The EOs of pennyroyal, spearmint or thyme have also been reported as repellents against *M. persicae* in laboratory conditions (Hori 1999b).

Citral, linalool, farnesol, or geraniol have been reported to exert repellent effects on *M. persicae* in laboratory choice bioassays (Gutiérrez et al. 1997; Gabryś et al. 2005; Dancewicz et al. 2010). *(Z)*-jasmone, a compound present in many EOs, is also considered as a repellent against the cereal aphid *Sitobion avenae* (Fabricius) and the lettuce aphid *Nasonovia ribisnigri* (Mosley) (Birkett et al. 2000; Bruce et al. 2003). As described in other studies, some EOs are toxic to aphids, like anise (*Pimpinella anisum*), cumin (*Cuminum cyminum*) or fennel (*Foeniculum vulgare*) when they are topically applied to *M. persicae* (Benelli et al. 2018; Pavela 2018).

The main objective of this work was to study the repellent effects of a selected group of EOs and pure compounds on *M. persicae* and *M. euphorbiae*. The EOs and pure compounds were selected in continuation of our previous work on the bird cherry-oat aphid, *Rhopalosiphum padi* (Pascual-Villalobos et al. 2017). We performed a two-choice bioassay in order to distinguish whether the repellence is produced through settling inhibition or by olfactory repulsion. We also provided behavioral data regarding the response of natural enemies to the products.

Materials and methods

Essential oils and pure compounds

Eight plant EOs were studied. Three from Apiaceae: aniseed (*Pimpinella anisum* L.), coriander (*Coriandrum sativum* L.), fennel (*Foeniculum vulgare* Miller); 3 from Lamiaceae: basil (*Ocimum basilicum* L.), peppermint (*Mentha piperita* L.), lavandin (*Lavandula* hybrid); 1 from Rutaceae: lemon (*Citrus limon* (L.) Burm. f); and 1 from Poaceae: lemongrass (*Cymbopogon flexuosus* (Nees ex Steud.) W. Watson). Also, 12 pure compounds were studied: phenylpropanoids (*(E)*-anethole), monoterpenic ketones (carvone), monoterpenic aldehydes (citral), monoterpene alcohols (geraniol and linalool), other alcohols (farnesol and *(Z)*-hexenol), monoterpenic hydrocarbons (limonene and γ-terpinene), and other products (caryophyllene, *(Z)*-jasmone and methyl salicylate). EOs were purchased from Distilleries Muñoz Gálvez S.A. (Murcia, Spain) and pure compounds from Sigma Aldrich. Lemongrass EO was obtained from plants grown at Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA) facilities (Murcia, Spain).

Insects

Aphids

Macrosiphum euphorbiae and two clones of *M. persicae*, clone 1 (red clone) and clone 2 (green clone) were used in bioassays. Clone 1 was collected in Campo de Cartagena (Murcia, Spain) from pepper plants (2016), clone 2 was collected in Orihuela (Alicante, Spain) from *Brassica oleracea* var. Italica (2016) and *M. euphorbiae* was collected from pepper plants in La Alberca (Murcia, Spain) (2017). All aphid species were maintained on pepper plants (*Capsicum annuum* L., cv Herminio) in a growth chamber under a 16:8 (L:D) photoperiod, at 25 ± 1 °C and 65% relative humidity for several generations prior to the performance of the assays. Adult aphids of the same size were used in all the bioassays.

Natural enemies

Larvae of the predator *S. rueppellii* were obtained from BioNostrum Pest Control S.L. (Alicante, Spain). Freshly received second-instar larvae (five-six days-old) were used in all the bioassays.

The parasitoid *A. colemani* was obtained from Agrobio S.L. (Almería, Spain) as mummies. Mummies were reared in ventilated plastic containers (0.89 L) and maintained in a growth chamber under a 16:8 (L:D) photoperiod, at 22 ± 1 °C and 60 ± 10% RH. Female adults were fed with cotton pads soaked in 30% (v/v) honey solution in water. One- to three-day-old female adults that emerged from mummies were used in the bioassays.

Two-choice bioassay (Aphid settling inhibition)

An aphid settling inhibition bioassay was performed in square plastic Petri dishes of 3 × 3x1.5 cm, provided with a mesh-covered air vent (1 cm diameter), according to a previously published method (Gutiérrez et al. 1997). Each box contains two halves (control and treatment) of pepper leaf discs (1 cm^2) (*C. annuum*, cv. Herminio) placed on agar at 1.15% in the box lid. In the initial screening, EOs and pure compounds were diluted in 99% acetone (Sigma Aldrich Company, St. Louis, MO, USA) at 2% (v/v) and applied to the pepper leaf using a pipette (10 µl/cm^2) (0.2 µl of EO or pure compound per cm^2) and led dry 2 min. The control was treated with acetone. Twenty boxes with 10 wingless adults in each one were included in each treatment. Aphids were released in the inside of the lid of the Petri dishes and then they were sealed. Boxes were maintained under controlled conditions at 22 ± 2 °C and 16:8 h (L:D) photoperiod. The Repellence Index (%) (*RI*) was calculated after 24 h using the formula (Gutiérrez et al. 1997):

$$RI(\%) = \left(1 - \frac{T}{C}\right) \times 100$$

T is the number of aphids on the treated surface and C is the number of aphids located in the control.

For those products giving a $RI \geq 70$, repellent doses 50 and 90 (RD_{50} and RD_{90}, respectively) were also calculated. The dose of solutions ranged were between 0.125 and 4% (v/v). A minimum of 4 doses was used to calculate the repellent dose for each product. RD_{50} and RD_{90} values were compared among products for each aphid species and among aphid species for each product.

Olfactometer bioassays

The bioassays were performed on a two-way olfactometer, as described by Vaello et al. (2017). The olfactometer consists of a Y-shaped tubular piece of Pyrex glass with an inner diameter of 15 mm, a 60 mm stem and two arms of 50 mm put together with an angle of 60° (Pobel, Madrid, Spain). Each arm was connected to a 250 ml Drexler bottle which contain a filter paper disc (2 cm diameter) as the odour source. All connections were made with Teflon tubes. The filter paper in one bottle was moistened with 10 µL of the product to be tested. The control was untreated filter paper. *(E)*-anethole, farnesol, *(E)*-anethole + farnesol (1:1), and *(Z)*-jasmone were the treatments tested on wingless and winged adults aphids of two species: *M. persicae* (clone 1) and *M. euphorbiae*. The same compounds together with the addition of aniseed EO, citral, and methyl salicylate were tested on adult females of *A. colemani* and *S. rueppellii* larvae. A flow of 700 ml min^{-1} of previously activated charcoal-filtered air was passed through each olfactometer arm. Air flow was controlled using a flow meter (Cole-Parmer, Vernon Hills, Illinois). All olfactometer tests were performed at a temperature of 25 ± 2 °C. In order to achieve a uniform light exposure and avoid any visual stimulation during the experiments, the olfactometer was placed into a black box opened in the top (Fig. 1). A led lamp was placed on the top providing a luminous emittance around 35 µmol·m^{-2} s^{-1}, except for *S. rueppellii* which was performed in the dark. All olfactometer tests were performed between 9 am and 6 pm. Each insect had 5 min to make a choice, except for *S. rueppellii* larvae, which had 3 min. A choice made by aphids was considered if they positioned themselves 3 cm above the bifurcation, whereas for *S. rueppellii* larvae, this was 2 cm. Two choices were recorded for *A. colemani*, the first when is positioned themselves 3 cm above the intersection and second choice after 5 min or when the insect was in the end of the arm (Du et al. 1998). Insects that did not make a choice within the established time were noted as non-choice and were excluded from the statistical analysis. For each product, 120 insects were individually tested in six groups of 20. A minimum choice of 40% was considered for *S. rueppellii* larvae in each group, and 60% for aphids and parasitoids, based on insect mobility and previous literature (Du et al. 1998; Dardouri et al. 2019). The y-tube glass was renewed every five insects, and the positions of the sources

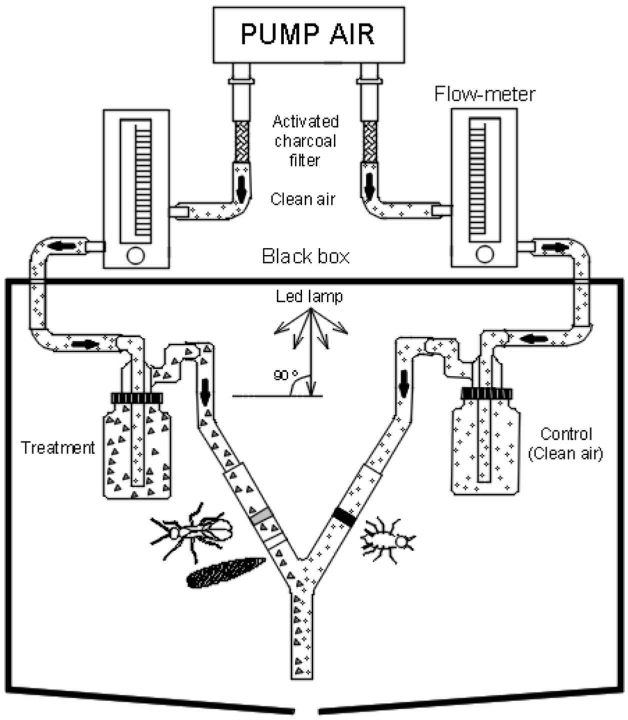

Fig. 1 Y-tube olfactometer to assess the behavioral response of predator *Sphaerophoria rueppellii*, parasitoid *Aphidius colemani* or aphids (wingless or winged), *Myzus persicae* or *Macrosiphum euphorbiae*. Six replications of 20 insects for each product was tested individually. Air flow was filtered with activated charcoal and regulated by flow meter to 700 mL min-1. Each insect chooses if is positioned in the election zone during 5 min, except for *Sphaerophoria rueppellii*, which had 3 min

were changed in order to avoid any bias in the choice. Source treatments were renewed every 10 insects. All glassware was washed with soapy water, rinsed in distilled water and finally in 99% acetone and then dried.

Toxicity bioassays

Contact toxicity (in aphids)

The toxic effect of the most active pure compounds in the two-choice and olfactometer bioassays was studied as described by Ribeiro et al. (2014). Pepper plants (*C. annuum*, cv. Herminio) 45–50 days old (6–8 leaves) in pots (0.33L) were cultivated on a mixture of peat (Klasmann TS3, Klasmann-Deilmann GmbH, Germany) and vermiculite (3:1) and were watered 2 times a week, once with mixture fertilizer of NPK (15–15-15) diluted in water. Plants were infested with 10 wingless adults of *M. persicae* (clone 1) or *M. euphorbiae* with a fine brush. Infested plants were maintained at 25 ± 1 °C and 16:8 (L:D) photoperiod for 5 days to rear the colony. After this time, the number of aphids per plant was counted. Plants were treated with nanoemulsions of *(E)*-anethole, farnesol, *(E)*-anethole + farnesol (1:1) and *(Z)*-jasmone at a dose of 0.2% (v/v) and Tween 80® (Panreac, chemical pure (pharma grade)) as surfactant (1:2) prepared following the procedure of previous studies (Pascual-Villalobos et al. 2017). A volume of 50 ml per plant was sprayed with a manual atomizer (Berry 1.5, Matabi, Goizper Group, Gipuzkoa, Spain). The concentration used (0.2% (v/v)) was determined based on previous observation (Pascual-Villalobos et al. 2019). Ten plants were used per treatment. Each group of plants was kept in a different growth chamber (Sanyo MLR-350) throughout the experiment.

Surviving aphids per plant were counted at 24, 48, 72, 96, and 168 h post-treatment. The instantaneous population growth ratio (*ri*) was calculated using the formula described by Stark and Banks (2003):

$$ri = \frac{\ln \frac{Nf}{No}}{\Delta T}$$

Nf is the number of aphids on each day after treatment, *No* is the initial number of aphids and ΔT are the days between both counts.

Likewise, control efficacy was calculated according to the Henderson and Tilton (1955) formula.

Residual toxicity (in natural enemies)

The residual effect of some pure compounds (*(Z)*-jasmone, *(E)*-anethole, farnesol and *(E)*-anethole+farnesol (1:1)) on female adults of *A. colemani* and larvae of *S. rueppellii* was studied following a standardized protocol from the International Organization for Biological Control (IOBC) (Mead-Briggs et al. 2000). Nine cm diameter Petri dishes were used in trials with *S. rueppellii*, while wooden boxes with 60 cm^2 lateral glass (10 × 6 cm) were used for female adults of *A. colemani*. The glass contact surface was treated with a computer-controlled spraying apparatus (Burkard Manufacturing Co. Ltd., England). The products tested, which are described above, were formulated as o/w nanoemulsions following the procedure previously described (Pascual-Villalobos et al. 2017), at concentrations ranging from 0.1–0.3% (v/v) that are likely to be used for spraying aphid infected plants under field conditions. Tween 80® was used as a surfactant at a ratio of 1:2. For each group, the Tween 80® concentration in the highest dose (*(E)*-anethole+farnesol (1:1)) was used as a control. *A. colemani* adults were fed with a 30% (v/v) honey solution and *S. rueppellii* larvae with 50 aphids per larva and day. The number of dead insects was recorded after 24 and 48 h. The glass was washed with soapy water, rinsed in distilled water and finally in 99% acetone and then dried. New Petri dishes were used in each trial.

Each treatment consisted of four replicates with 10 adults of *A. colemani* and eight replicates of 5 larvae of *S. rueppellii* for each treatment and concentration.

Statistical analysis

Data of the aphid settling inhibition bioassay were analyzed using "R" software, version 3.6.2 (R Core Team 2019). Repellent doses RD_{50} and RD_{90} of the two-choice bioassay were estimated from log-normal dose–response model, calculated with drm function from R package drc (Ritz et al. 2015). Different aphid species were compared with the edcomp function to compare the relative potencies between dose–response curves.

Data from the Y-tube olfactometer bioassay were analyzed to test whether volatile products had a significant effect compared to the untreated control. Exact two-sided binomial test and Clopper–Pearson 95% confidence intervals calculated with binom. test, from R package stats.

The data obtained in the contact toxicity bioassay was analyzed by a one-way ANOVA and the means were separated by Fisher's LSD test. Normality of the data and homogeneity of variance was assessed with Shaphiro–Wilk and Levene's tests, respectively. The data were analyzed using Statgraphics (Centurion 18.1.6.).

Mortality data from residual toxicity in the natural enemies bioassay was pairwise compared within each natural enemy, dose, and time, using the two-tailed Fisher's exact test with Holm–Bonferroni p value adjustment, using R package the R function *pairwise.* fisher. test from package fmsb (Nakazawa and Nakazawa 2019).

Results

Aphid settling inhibition in *Myzus persicae* and *Macrosiphum euphorbiae*

The lowest values of the repellency index (*RI*) were obtained for the lemon EO ($RI = 37.8$) and for its main compound limonene ($RI = 15.0$) meaning absence of repellency ($RI < 70\%$). Five out of eight EOs tested were effective against *M. persicae* (clone 1), while six out of eight gave repellence in *M. persicae* (clone 2). Only three out of eight EOs were effective on *M. euphorbiae*. Aniseed, basil and lemongrass EOs were active ($RI \geq 70$) causing repellence both in *M. persicae* (in the two clones studied) and in *M. euphorbiae*, the most effective one being aniseed EO. Strikingly, coriander, peppermint, fennel, and lavandin EOs proved effective against one clone of *M. persicae* but not the other. Pure compounds presented the highest *RI* in the bioassay. *(E)*-anethole ($RI = 98.1$) is the most repellent for clone 1 of *M. persicae;* geraniol ($RI = 96.9$) and *(Z)*-jasmone ($RI = 94.5$) for clone 2, whereas geraniol ($RI = 99.4$) and farnesol ($RI = 94.3$) for *M. euphorbiae*.

RD_{50} and RD_{90} in Tables 1 and 2 were calculated for those EOs and pure compounds active in the initial screening. Only aniseed, basil, and lemongrass EOs affected both species of aphids. These EOs had the most effective RD_{50} (ranging from the $RD_{50} = 0.039$ µl/cm^2 of lemongrass EO against *M. euphorbiae*, to the $RD_{50} = 0.110$ µl/cm^2 shown by basil EO against *M. persicae*, clone 2). However, other EOs only affected *M. persicae*. Peppermint and coriander affected both clones, lavandin EO only affected clone 2, whereas fennel only repelled clone 1 of *M. persicae* (Table 1).

Regarding pure compounds (Table 2), seven out of the twelve compounds tested gave a $RI \geq 70$ against *M. persicae* (clone 1), six against *M. persicae* (clone 2) and seven against *M. euphorbiae*. Five compounds, *(E)*-anethole, citral, geraniol, farnesol, and *(Z)*-jasmone, were effective against all aphid species included in the study. Carvone was effective against *M. persicae* but not against *M. euphorbiae*, while caryophyllene showed repellence against *M. euphorbiae* but not against *M. persicae* (Table 2). Linalool was repellent against *M. persicae* (clone 1) and *M. euphorbiae*, but not against clone 2.

Also, significant differences were produced between aphid species or even aphid clones for a given product if we compare RD_{50} and RD_{90} (Table 3). According to the RD_{50} values, this effect was different for the two clones of *M. persicae* in all EOs, except peppermint ($P > 0.05$). Clone 2 of *M. persicae* and *M. euphorbiae* were more susceptible to lemongrass EO, and clone 2 to aniseed. Farnesol, geraniol, and *(E)*-anethole were more effective against clone 2 of *M. persicae* than for other aphids, whereas *M. euphorbiae* was more susceptible to citral, farnesol, and linalool, than clone 1. In summary, clone 1 of *M. persicae* was the most resistant aphid, less affected by products than other aphids.

Effect of compounds on the olfactory response of insects

(E)-anethole, farnesol, the mixture of both (1:1), and *(Z)*-jasmone were selected to perform the olfactometry bioassays because they were the most repellent products against aphids in the previous bioassay. The results of the olfactometry are plotted in Figs. 2,3,4,5 (aphids) and Figs. 6 and 7 (natural enemies).

Aphids

None of the compounds tested gave a significant response on *M. persicae* wingless adults (Fig. 2), and only farnesol was repellent ($P = 0.012$) for *M. persicae* winged adults (Fig. 3). Likewise, farnesol and *(Z)*-jasmone induced repellency on wingless adults ($P = 0.001$; $P < 0.0001$, respectively), and

Table 1 Repellent activity of essential oils against two clones of *Myzus persicae* and *Macrosiphum euphorbiae* in choice bioassays after 24 h

	Myzus persicae (clone 1)			*Myzus persicae* (clone 2)			*Macrosiphum euphorbiae*		
	R.I. (%)[a]	RD_{50}[b]	RD_{90}[c]	R.I. (%)[a]	RD_{50}[b]	RD_{90}[c]	R.I. (%)[a]	RD_{50}[b]	RD_{90}[c]
Aniseed	96.5	0.072e (0.063–0.081)	0.390a (0.313–0.468)	91.9	0.060d (0.055–0.065)	0.139b (0.123–0.155)	88.3	0.081ª (0.075–0.088)	0.210b (0.178–0.241)
Basil	93.9	0.087d (0.082–0.092)	0.159c (0.147–0.170)	83.7	0.110b (0.103–0.118)	0.220ª (0.200–0.241)	90.7	0.085ª (0.075–0.094)	0.383ª (0.295–0.471)
Coriander	64.9	0.184ª (0.171–0.196)	_[d]	90.6	0.128a (0.113–0.144)	_[d]	61.8	–	–
Fennel	74.8	0.132b (0.118–0.146)	_[d]	61.8	–	–	33.3	–	–
Lemon	37.8	–	–	64.2	–	–	60.7	–	–
Lemongrass	82.5	0.100c (0.092–0.107)	0.290b (0.256–0.324)	92.1	0.047e (0.041–0.053)	0.208ª (0.167–0.248)	82.7	0.039b (0.032–0.046)	0.285ª (0.213–0.358)
Peppermint	91.1	0.101c (0.093–0.108)	_[d]	77.0	0.093c (0.079–0.106)	_[d]	49.6	–	–
Lavandin	60.5	–	–	82.8	0.084c (0.076–0.091)	0.237ª (0.200–0.274)	68.7	–	–

[a]Repellency Index (*R.I.*) = [1 − (T/C)] × 100 where T = total number of aphids on treated leaf after 24 h and C = total number of aphids on control leaf after 24 h (Gutierrez et al. 1997); replications with less than 40% of aphid settlement were discarded for computation; EOs were applied at doses of 0.2 µl/cm^2

[b,c]RD_{50} and RD_{90} are doses of compounds (µl/cm^2) that give *R.I.* values of 50 and 90, respectively. Doses were calculated in active products only ($RI \geq 70$ at 0.2 µl/cm^2). RD_{50} and RD_{90} are followed by letters to indicate differences among treatments for each aphid. A 95% confidence interval was calculated. All calculations were made using the R package DRC (Ritz et al. 2015)

[d]Values of RD_{90} interval are above the highest concentration used

winged adults ($P = 0.027$; $P < 0.0001$, respectively) of *M. euphorbiae* (Figs. 4 and 5). Although none of the aphid species exhibited a significant response to *(E)*-anethole and the mixture of *(E)*-anethole+farnesol (1:1) (Figs. 2,3,4,5) in olfactometer tests.

Natural enemies

Farnesol was attractive to larvae of *S. rueppellii* ($P < 0.01$) (Fig. 6) and female adults of *A. colemani* ($P < 0.0001$) (Fig. 7). Aniseed EO ($P = 0.011$) and its main compound *(E)*-anethole ($P < 0.0001$) had a significant repellent effect on larvae of *S. rueppellii* (Fig. 6), whereas citral ($P < 0.0001$), *(Z)*-jasmone ($P < 0.0001$) and methyl salicylate ($P = 0.001$) were repellents to *A. colemani* (Fig. 7).

Toxicity

Contact toxicity in aphids

The plants used for the experiments presented equal initial populations of *M. persicae* (clone 1) or *M. euphorbiae*, counted as the average number of aphids per plant (± 65 and ± 35, respectively) ($P > 0.05$) (Table 4) and were therefore considered suitable for the assay. Treatments at 0.2% (v/v) of essential oils were no produce damages to the plants.

In both species of aphids, spraying the nanoemulsions of farnesol and *(E)*-anethole+farnesol (1:1) reduced the population of insects at 96 h post-treatment in *M. euphorbiae* ($ri = -0.16$ and -0.21, respectively) and 72 h post-treatment in *M. persicae* ($ri = -0.12$ and -0.13, respectively). At 168 h post-treatment, the average number of aphids was higher than initially but much lower than the control in *M. persicae* ($F_{(4,45)} = 22.58$, $P = 0.000$) and *M. euphorbiae* ($F_{(4,45)} = 5.24$, $P = 0.0015$) (Table 4).

(E)-anethole causes a reduction in the population at 72 h ($ri = -0.10$) and pauses the development of the colony at 168 h ($ri = 0.14$) in *M. euphorbiae*. *(Z)*-jasmone also slowed down the development of the colonies of *M. persicae* compared with the control at 48 h ($P = 0.012$), although it does not cause a significant reduction in the number of aphids per plant (Table 4).

The binary mixture of *(E)*-anethole + farnesol (1:1) gave a high reduction in the instantaneous rate of population growth of *M. persicae* ($ri = -0.78$) and *M. euphorbiae* ($ri = -3, 85$) at 24 h, although, at 168 h, the ratios of farnesol and *(E)*-anethole+farnesol (1:1) are similar for *M. persicae* (0.19 and 0.18, respectively) and *M. euphorbiae* (0.03 and 0.02, respectively) (Table 4).

Table 2 Repellent activity of pure compounds against two clones of *Myzus persicae* and *Macrosiphum euphorbiae* in choice bioassays after 24 h

	Myzus persicae (clone 1)			*Myzus persicae* (clone 2)			*Macrosiphum euphorbiae*		
	R.I. (%)[a]	RD_{50}[b]	RD_{90}[c]	R.I. (%)[a]	RD_{50}[b]	RD_{90}[c]	R.I. (%)[a]	RD_{50}[b]	RD_{90}[c]
(E)-anethole	98.1	0.065d (0.060–0.070)	0.152d (0.136–0.168)	85.8	0.042b (0.036–0.047)	0.186ab (0.155–0.217)	90.3	0.086b (0.076–0.095)	_[e]
Carvone	94.0	0.109c (0.103–0.115)	0.179c (0.167–0.192)	83.6	0.094[a] (0.088–0.100)	0.210[a] (0.185–0.236)	39.5	–	–
Caryophyllene	41.7	–	–	42.9	–	–	72.8	0.181[a] (0.162–0.200)	_[e]
Citral	74.8	0.126b (0.118–0.134)	0.277b (0.249–0.305)	79.8	_[d]	0.153b (0.121–0.185)	97.1	0.029e (0.026–0.033)	0.126b (0.102–0.150)
Geraniol	87.3	0.057d (0.049–0.065)	0.331ab (0.258–0.404)	96.9	0.034c (0.030–0.037)	0.117b (0.101–0.132)	99.4	0.040d (0.036–0.044)	0.141b (0.118–0.164)
Farnesol	87.6	0.037e (0.033–0.041)	0.126d (0.108–0.145)	91	_[d]	0.115b (0.082–0.149)	94.3	0.022f (0.019–0.025)	0.093c (0.077–0.108)
(Z)-Hexenol	59.5	–	–	18.7	–	–	29.0	–	–
(Z)-Jasmone	91.3	0.011f (0.008–0.015)	0.142 cd (0.106–0.178)	94.5	_[d]	0.039c (0.025–0.053)	92.7	0.013 g (0.007–0.019)	0.123bc (0.083–0.162)
Limonene	15.0	–	–	32.4	–	–	19.8	–	–
Linalool	76.4	0.192[a] (0.182–0.203)	0.386[a] (0.350–0.421)	15.8	–	–	82.7	0.058c (0.051–0.065)	0.267[a] (0.214–0.319)
Methyl salicilate	44.6	–	–	55.1	–	–	64.8	–	–
γ-Terpinene	63.8	–	–	61.0	–	–	55.7	–	–

[a]Repellency Index (R.I.) = [1-(T/C)] × 100 where T = total number of aphids on treated leaf after 24 h and C = total number of aphids on control leaf after 24 h (Gutierrez et al. 1997); replications with less than 40% of aphid settlement were discarded for computation; EOs were applied at doses of 0.2 µl/cm^2

[b,c]RD_{50} and RD_{90} are doses of compounds (µl/cm^2) that give *RI* values of 50 and 90, respectively. Doses were calculated in active products only ($RI \geq 70$ at 0.2 µl/cm^2). RD_{50} and RD_{90} are followed by letters to indicate significant differences among treatments for each aphid. A 95% confidence interval was calculated. All calculations are made using the R package DRC (Ritz et al. 2015)

[d]Values of RD_{50} interval are below the lowest concentration used

[e]Values of RD_{90} interval are above the highest concentration used

Residual toxicity in natural enemies

The compounds were formulated as nanoemulsions ranging from 0.1 to 0.3% (v/v) (Table 5). Controls with water were carried out in each of the trials, resulting in a mortality of less than 10%.

There were no relevant side effects on the larvae of *S. rueppellii* (Table 5). No significant differences were found between products at the high doses ($P > 0.05$). The mixture of *(E)*-anethole+farnesol (1:1) showed a slight toxicity, with mortalities of 37.5 and 20% at 48 h for 0.1 and 0.3%, respectively. However, mortality values were lower (usually < 10%) when the pure compounds were applied alone (Table 5). Blending might favour penetration of the compounds through the insect cuticle and, therefore, the toxic effects.

Aphidius colemani was more sensitive to pure compounds than *S. rueppellii* (Table 5). The treatment with 0.1% *(Z)*-jasmone doubled the mortality recorded with *S. rueppellii* at 48 h, while at a dose of 0.2%, mortality increased to around 75%. High mortality (75,7%) was also recorded in the treatment with 0.1% farnesol at 48 h ($P = 0.0047$), that reached 100% when farnesol was applied at 0.2%. In the case of *(E)*-anethole, the mortality recorded was 37.1% when applied at 0.1% and again 100% at 0.2%. The mixture of *(E)*-anethole and farnesol also gave mortalities above 75% at the lower dose and 100% when each compound was applied at 0.2% (Table 5). It is worth noting, finally, the high values of mortality recorded for *A. colemani* in the respective controls used (Table 5).

Discussion

From our work, it can be concluded that some of the EOs studied (aniseed, basil and lemongrass) and natural compounds (*(E)*-anethole, farnesol, *(Z)*-jasmone, citral and geraniol) displayed clear repellent effects against the aphids of vegetables crops, *M. persicae* and *M. euphorbiae*. In our previous studies, a similar effect of these natural compounds was observed against the cereal aphid *R.*

Table 3 Comparison of repellent activity between the aphids, *Myzus persicae and Macrosiphum euphorbiae*, in each essential oil or pure compound

Essential oils	Aniseed	Basil	Coriander	Lemongrass	Peppermint		
RD_{50}[a]							
Myzus persicae (clone 1)	0.072a	0.087b	0.184a	0.100a	0.101a		
Myzus persicae (clone 2)	0.060b	0.110a	0.128b	0.047b	0.093a		
Macrosiphum euphorbiae	0.081a	0.085b	–	0.039b	–		
RD_{90}[a]							
Myzus persicae (clone 1)	0.390a	0.159c	–	0.290a	–[c]		
Myzus persicae (clone 2)	0.139c	0.220b	–[c]	0.208b	–[c]		
Macrosiphum euphorbiae	0.210b	0.383a	–[c]	0.285ab	–		
Pure compounds	*(E)*-anethole	Carvone	Citral	Geraniol	Farnesol	*(Z)*-jasmone	Linalool
RD_{50}[a]							
Myzus persicae (clone 1)	0.065b	0.109a	0.126a	0.057a	0.037a	0.011a	0.192a
Myzus persicae (clone 2)	0.042c	0.094b	–[b]	0.034c	–[b]	–[b]	–
Macrosiphum euphorbiae	0.086a	–	0.029b	0.040b	0.022b	0.013a	0.058b
RD_{90}[a]							
Myzus persicae (clone 1)	0.152b	0.179b	0.277a	0.331a	0.126a	0.142b	0.386a
Myzus persicae (clone 2)	0.186a	0.210a	0.153b	0.117b	0.115ab	0.039a	–
Macrosiphum euphorbiae	–[c]	–	0.126b	0.141b	0.093b	0.123b	0.267b

[a]Repellent doses 50 and 90 (RD_{50} and RD_{90}) values (without confidence interval) followed by different letters in the columns indicate significant differences between aphids species or aphids clones. A 95% confidence interval was calculated. All calculations were made using the R package DRC (Ritz et al. 2015)

[b]Values of RD_{50} interval are below the lowest concentration used. RD_{50} could not be compared with others

[c]Values of RD_{90} interval are above the highest concentration used. RD_{90} could not be compared with others

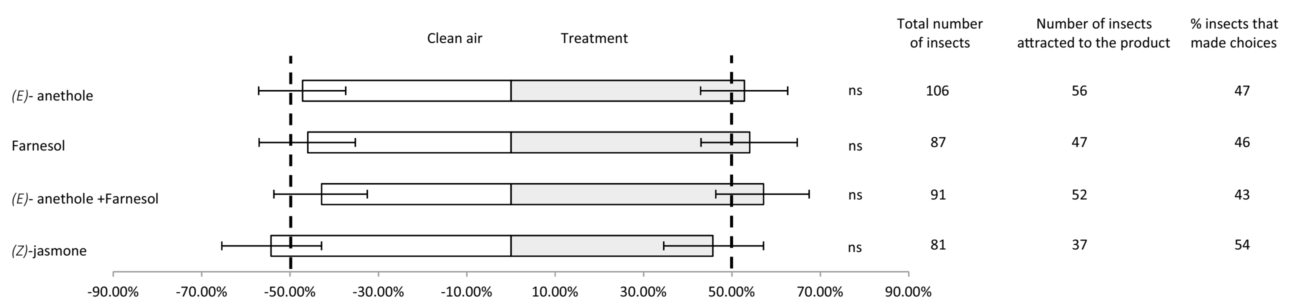

Fig. 2 Olfactometer (y-tube) response (%) of *Myzus persicae* (clone 1) wingless adult females (6 replications of 20 aphids each) to volatile products (10 µl individually or 20 µl (10+10) in binary mixture) after 5 min. Statistical significance according to Exact two-sided Binomial test (*P < 0.05; ***P* < 0.01; ****P* < 0.001; ns *P* > 0.05) and Clopper-Pearson 95% confidence intervals

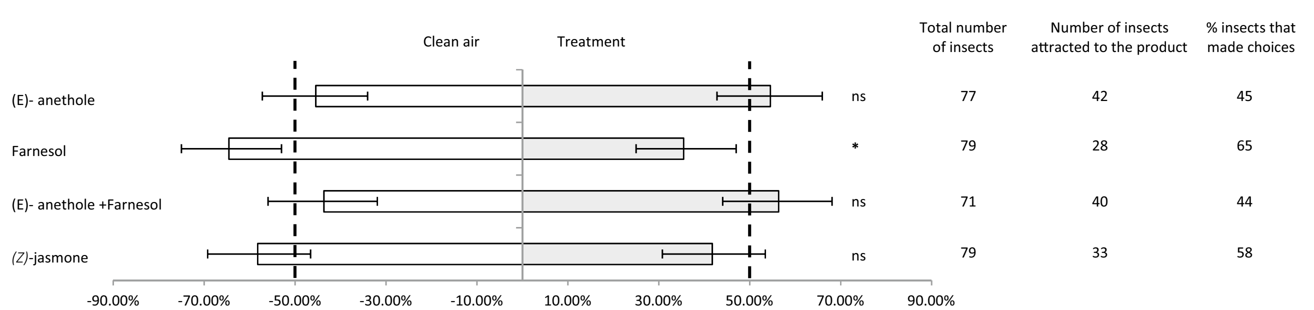

Fig. 3 Olfactometer (y-tube) response (%) of *Myzus persicae* (clone 1) alate adult females (6 replications of 20 aphids each) to volatile products (10 µl individually or 20 µl (10 + 10) in binary mixture) after 5 min. Statistical significance according to Exac two-sided Binomial test (**P* < 0.05; ***P* < 0.01; ****P* < 0.001; ns *P* > 0.05) and Clopper–Pearson 95% confidence intervals

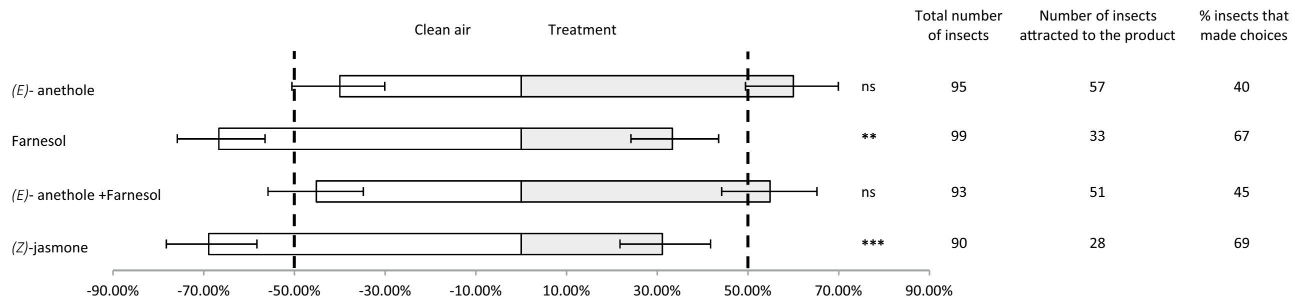

Fig. 4 Olfactometer (y-tube) response (%) of *Macrosiphum euphorbiae* wingless adult females (6 replications of 20 aphids each) to volatile products (10 µl individually or 20 µl (10+10) in binary mixture) after 5 min. Statistical significance according to Exact two-sided Binomial test (*$P<0.05$; **$P<0.01$; ***$P<0.001$; ns $P>0.05$) and Clopper–Pearson 95% confidence intervals

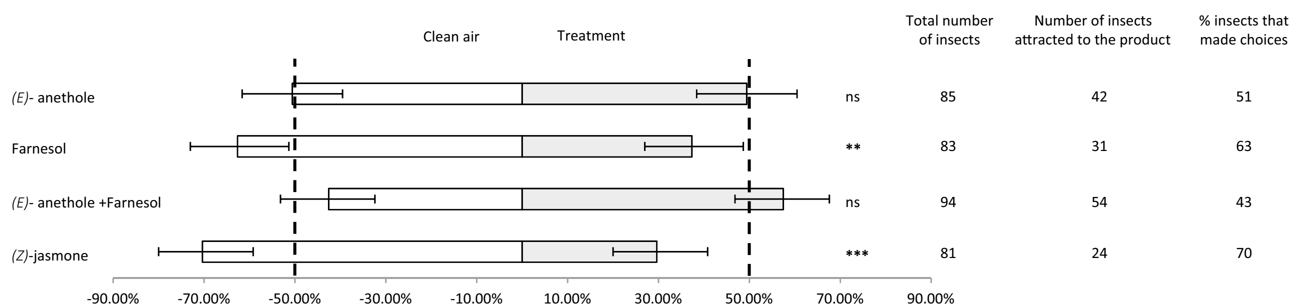

Fig. 5 Olfactometer (y-tube) response (%) of *Macrosiphum euphorbiae* alate adult females (6 replications of 20 aphids each) to volatile products (10 µl individually or 20 µl (10+10) in binary mixture) after 5 min. Statistical significance according to Exac two-sided Binomial test (*$P<0.05$; ** $P<0.01$; ***$P<0.001$; ns $P>0.05$) and Clopper–Pearson 95% confidence intervals

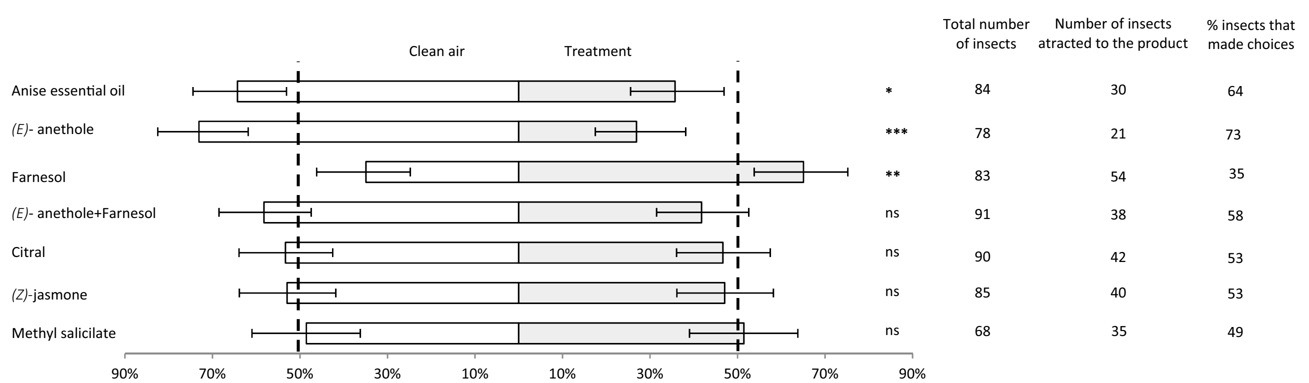

Fig. 6 Olfactometer (y-tube) response (%) of *Sphaerophoria rueppellii* L2 larvae (6 replications of 20 larvae each) to volatile products (10 µl individually or 20 µl (10+10) in binary mixture) after 5 min. Statistical significance according to Exact two-sided Binomial test (*$P<0.05$; **$P<0.01$; *** $P<0.001$; ns $P>0.05$) and Clopper–Pearson 95% confidence intervals

padi. However, basil, coriander, fennel and peppermint were not repellent to *R. padi* (Pascual-Villalobos et al. 2017).

The results of the olfactometer refer to an olfactive repellence in which a volatile action occurs. However, the repellency of the aphid settling inhibition assay refers more properly to a deterrence (Isman, 2019) in which it is not possible to differentiate its nature (antifeedant or other).

Some works show the toxic effect through contact of the anise and fennel EOs (Benelli et al. 2018; Pavela 2018) or by fumigant activity (Tunç and Şahinkaya 1998; Digilio et al. 2008) to aphids. Our results show a settling inhibition from

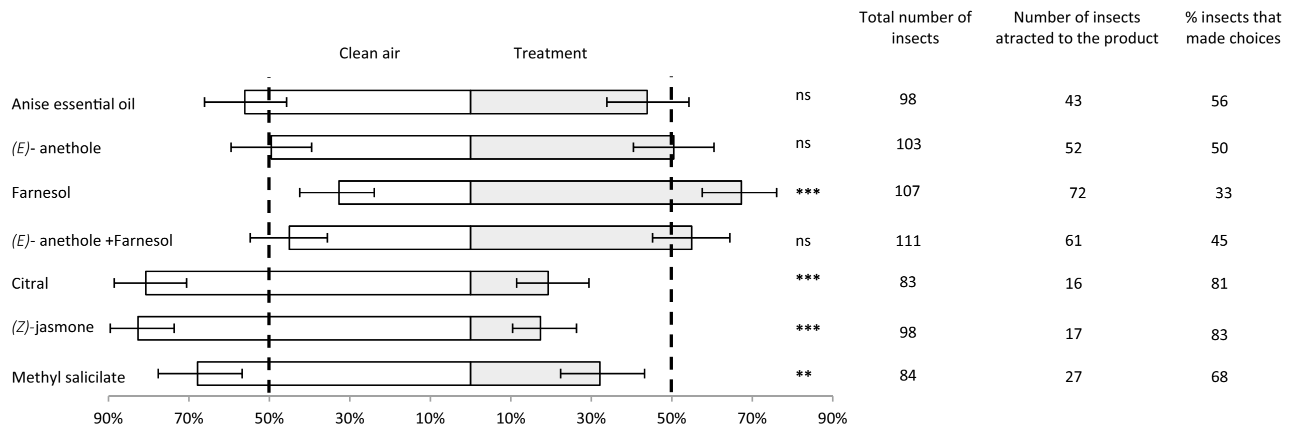

Fig. 7 Olfactometer (y-tube) response (%) of *Aphidius colemani* female adults (6 replications of 20 insects each) to volatile products (10 µl individually or 20 µl (10+10) in binary mixture) after 5 min. Statistical significance according to Exact two-sided Binomial test (*$P<0.05$; **$P<0.01$; ***$P<0.001$; ns $P>0.05$) and Clopper–Pearson 95% confidence intervals

(E)-anethole and aniseed EO. However, in the olfactometry assay, *(E)*-anethole does not act as an olfactive repellent for *M. persicae* and *M. euphorbiae*. For this reason, we suggest that the repellency attributed to EO rich in *(E)*-anethole and the compound described in this study and in previously published papers (Pascual-Villalobos et al. 2017, 2019, 2020) is due to a non-olfactive repellent effect (produced by a residual toxicity). Faraone et al. (2015) observed repellency against *M. persicae* when exposed to cabbage leaf discs treated previously with linalool, but no mortality was produced. However, the topical application of linalool on *M. persicae* causes mortality. This repellence may occur because linalool is toxic, but no produce mortality when insect is not exposed directly. In our results, the case of *(E)*-anethole is similar, because in our previous works we prove that mortality is produced in *M. persicae* (Pascual-Villalobos et al. 2020).

It can be proposed that aphids cannot perceive volatiles from *(E)*-anethole until it remains exposed for a long time or is in contact with it (even if it is residual). As for coriander and fennel, they have no effect against *M. euphorbiae*, while they do for *M. persicae*, despite the fact that one of the main compounds in fennel is *(E)*-anethole and this affected to all of them.

The highest repellency was obtained for the pure compounds, rather than the EOs. According to Deletre et al. (2016), the repellent effect of EOs results from one or several major compounds. In our results, this happens in the case of aniseed, which is less effective than its main compound, *(E)*-anethole, or lemongrass and its main compound, citral. This may be due to an antagonistic effect produced by other compounds present in the EO. For instance, Bruce et al. (2005) conclude that the repellent effect of *(E)*-β-farnesene (the main compound of the EO of *Hemizygia petiolata*) against *M. persicae* was lower than expected as a consequence of the presence of minor components in the oil, (+)-bicyclogermacrene and (−)-germacrene D.

Lemongrass EO and its main compound citral are repellent for vegetable aphids. Citral is a mixture of geranial + neral isomers and is reported to be repellent and deterrent for insects (Leal and Uchida 1998; Lü and Liu 2016; Plata-Rueda et al. 2020). In a two-choice bioassay, Gabryś et al. (2005) obtained a significant effect against *M. persicae* with ethanolic solutions of citral at 0.1% (v/v). Previously working on *R. padi* (Pascual-Villalobos et al. 2017), a high repellency was obtained with citral. Geraniol is usually one of the major compounds in the EOs of citronella, geranium and roses (Tamagawa-Mineoka et al. 2007). In our results, it is one of the most repellent compounds in the two-choice bioassay. Gutiérrez et al. (1997) also obtained a repellent effect of geraniol on *M. persicae*. However, they obtained an RD_{50} of 47.13 µg/cm^2, equivalent to 0.0531 µl/cm^2 similar to the RD_{50} obtained in the present work (between 0.030 and 0.051 µl/cm^2 for all aphids), or in our previous work with *R. padi* with a RD_{50} of 0.044 µl/cm^2 (Pascual-Villalobos et al. 2017).

Farnesol and *(Z)*-jasmone are the compounds with the highest repellent effect in the two-choice bioassay. Farnesol repels the two forms of *M. euphorbiae*, but only winged adults of *M. persicae* in olfactometer bioassays are affected. This difference between wingless and winged adults of *M. persicae* may be explained by the type of antennal sensilla present in aphids. Nymphs and adults of aphids always have primary rhinarium (V and VI antennal segment) and winged forms can also have secondary rhinarium (III, IV and V antennal segments). In *M. euphorbiae*, both wingless and winged adults have primary and secondary rhinarium, while in the case of *M. persicae*, wingless adults only have

Table 4 Aphid populations of *Myzus persicae* (clone 1) and *Macrosiphum euphorbiae* in pepper plants before and after (in hours) spraying with nanoemulsions

Product	Number of aphids/plants[a]						C.E. (%)[b]					Instantaneous rate of population growth (*ri*)[c]				
	Initial	24	48	72	96	168 h	24	48	72	96	168 h	24	48	72	96	168 h
Myzus persicae (clone 1)																
(*E*)-anethole 0.2%	68.3 ± 6.37a	76.5 ± 7.78a	98.7 ± 9.25a	142.4 ± 8.81[a]	185.7 ± 11.55a	> 400a	0	0	0	0	0	0.10 ± 0.05a	0.18 ± 0.03a	0.25 ± 0.02a	0.26 ± 0.03a	0.26 ± 0.02a
Farnesol 0.2%	54 ± 6.21a	33.8 ± 3.98c	22.3 ± 2.87c	37.8 ± 4.10c	71.1 ± 8.09c	197.4 ± 20.23c	26.56	58.21	54.01	38.02	35.56	− 0.47 ± 0.11c	− 0.46 ± 0.07b	− 0.12 ± 0.04b	0.07 ± 0.04b	0.19 ± 0.02b
(*E*)-anethole 0.2% + farnesol 0.2%	58.5 ± 6.38a	27.8 ± 3.61c	26.6 ± 4.81c	41.1 ± 6.23c	63.9 ± 9.74c	211.2 ± 24.85c	44.25	53.99	53.84	48.58	36.36	− 0.78 ± 0.12d	− 0.43 ± 0.08b	− 0.13 ± 0.05b	0.01 ± 0.03b	0.18 ± 0.02b
(*Z*)-jasmone 0.2%	65.3 ± 9.34a	56.3 ± 8.91b	48.3 ± 9.04b	55.3 ± 11.82c	88.9 ± 14.99c	326.6 ± 30.66b	0	25.16	44.36	35.91	11.83	− 0.19 ± 0.07b	− 0.26 ± 0.15b	− 0.14 ± 0.08b	0.06 ± 0.03b	0.24 ± 0.02b
Control (Tween80® 0.8%)	68.4 ± 9.61a	58.37 ± 5.40b	48.3 ± 9.05b	104.1 ± 8.54b	145.3 ± 10.33b	> 400a	–	–	–	–	–	− 0.08 ± 0.10ab	0.02 ± 0.04a	0.17 ± 0.04a	0.21 ± 0.03a	0.27 ± 0.02a
P value	0.6072 ns	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001						< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0063
Macrosiphum euphorbiae																
(*E*)-anethole 0.2%	33.2 ± 3.30a	18.9 ± 3.23b	48.3 ± 9.06b	25.2 ± 3.05b	35.6 ± 7.01a	89.6 ± 11.52a	27.88	20.29	25.42	17.48	6.31	− 0.65 ± 0.16a	− 0.23 ± 0.06a	− 0.10 ± 0.04a	− 0.01 ± 0.03a	0.14 ± 0.01a
Farnesol 0.2%	34.7 ± 3.16a	10.60 ± 2.11bc	48.3 ± 9.07bc	14.3 ± 2.50c	18.5 ± 1.77b	54.2 ± 9.95b	61.3	53.57	59.51	58.97	45.78	− 1.38 ± 0.25ab	− 0.50 ± 0.07b	− 0.33 ± 0.06b	− 0.16 ± 0.03b	0.03 ± 0.05b
(*E*)-anethole 0.2% + farnesol 0.2%	32.9 ± 3.68a	7.50 ± 1.75c	48.3 ± 9.08c	13.3 ± 3.17c	18.9 ± 4.07b	51.5 ± 11.68b	71.12	66.4	60.28	55.79	45.66	− 3.85 ± 2.23b	− 0.77 ± 0.15c	− 0.40 ± 0.10b	− 0.21 ± 0.08b	0.02 ± 0.05b
(*Z*)-jasmone 0.2%	32.9 ± 3.38a	33.00 ± 5.35a	48.3 ± 9.09a	33.4 ± 2.63ab	38.1 ± 7.10a	97.6 ± 13.83a	0	0	0.25	10.88	0	0.10 ± 0.08a	− 0.01 ± 0.04a	0.01 ± 0.02a	0.02 ± 0.03a	0.15 ± 0.02a
Control (Tween80® 0.8%)	39.4 ± 2.88a	31.10 ± 4.09a	48.3 ± 9.10a	40.1 ± 5.68a	51.2 ± 6.14a	113.5 ± 12.44a	–	–	–	–	–	− 0.31 ± 0.15a	− 0.12 ± 0.08a	− 0.03 ± 0.07a	0.05 ± 0.04a	0.14 ± 0.02a
P value	0.4667	< 0.0001	< 0.0001	< 0.0001	0.0005	0.0015						0.0620	< 0.0001	< 0.0001	0.0007	0.0064

[a]Means (± SE) of aphids/plants followed by different letters within the column indicate significant differences among the treatments (ANOVA followed by LSD test; $p > 0.05$; F value d.f. (4, 45)

[b]C.E.: control efficacy calculated by means of Henderson and Tilton (1955) formula

[c]Instantaneous rate of population growth (*ri*) = ln (Nf/No)/ΔT where Nf is the number of aphids on each day after treatment, No is the initially number of aphids and ΔT are the days between them. Means (± SE) of *ri* followed by different letters within the column indicate significant differences among the treatments (ANOVA followed by LSD test; $p > 0.05$; F value d.f. (4, 45)

Table 5 Residual contact toxicity of bioactive volatiles on natural enemies

Product % v/v	*S. rueppellii* (% larvae mortality)[a]		*A. colemani* (% adult mortality)[b]	
	24 h	48 h	24 h	48 h
(Z)-jasmone 0.1%	7.5ab	22.5ab	10.8b	56.8ab
(E)-anethole 0.1%	2.5b	10.0ab	12.5b	37.1b
Farnesol 0.1%	7.5ab	17.5ab	27.0ab	75.7a
(E)-anethole 0.1% + farnesol 0.1%	27.5a	37.5b	47.2a	78.8a
Control (tween80® 0.4%)	2.5 b	5.0a	17.5ab	35.0b
(Z)-jasmone 0.2%	5.0a	5.0a	28.2c	74.4b
(E)-anethole 0.2%	2.5a	7.5a	62.2b	94.4ab
Farnesol 0.2%	5.0a	10.0a	85.0ab	100.0a
(E)-anethole 0.2% + farnesol 0.2%	10.0a	10.0a	94.7a	100.0a
Control (tween80® 0.8%)	0.0a	5.0a	82.1ab	87.2ab
(Z)-jasmone 0.3%	7.5a	12.5a	–	–
(E)-anethole 0.3%	2.5a	7.5a	–	–
Farnesol 0.3%	2.5a	2.5a	–	–
(E)-anethole 0.3% + farnesol 0.3%	17.5a	20.0a	–	–
Control (tween80® 1.2%)	0.0a	2.5a	–	–

[a]Mean of Mortality (%) followed by different letters indicate significant differences between products within each natural enemy, dose, and time. Each dose value was calculated with eight replications with 5 larvae/rep. All calculations are made using R software

[b]Mean of Mortality (%) followed by different letters indicate significant differences between products within each natural enemy, dose, and time. Each dose value was calculated with 15 adults/rep. All calculations are made using R software

the primary rhinarium (Shambaugh et al. 1978). The absence of secondary rhinarium in wingless adults of *M. persicae* may be the reason why they are not able to perceive farnesol.

Plants emits *(Z)*-jasmone when they are exposed to damage to plant tissue and this also acts as an elicitor of plant defenses (Dewhirst et al. 2012). *(Z)*-jasmone produced settling inhibition in both aphid species in the two-choice bioassay, however, volatiles only repelled *M. euphorbiae*. Birkett el al. (2000) obtained a repellency on the lettuce aphid *N. ribisnigri* in 4-arm olfactometer with 1 µg of *(Z)*-jasmone. Some authors have tested external applications of *(Z)*-jasmone on plants such as *Arabidopsis thaliana* (L.) to induce repellent signals against *M. persicae*. *(Z)*-jasmone treatments act as precursors to other repellent compounds (Bruce et al. 2008). However, these treatments are limited by the amount of compound that they are capable of releasing. Wheat plants treated with *(Z)*-jasmone showed repellency against *S. avenae* (Bruce et al. 2003), whereas repellency towards *M. persicae* in pepper plants treated with *(Z)*-jasmone could not be concluded (Dewhirst et al. 2012).

Farnesol is described as a juvenile hormone of *Tenebrio* (Coleoptera: Tenebrionidae), *Rhodnius* (Hemiptera: Reduviidae), and other insects (Wigglesworth 1963); as a sex pheromone of mites emitted by deutonymphs (Sonenshine 1985); and also as an ant repellent (Shorey et al. 1996); or honeybee attractant (Williams et al. 1981). Our results show a clear olfactive repellence and settling inhibition in aphids. Few works have studied the effect of farnesol on aphids, using *M. persicae* as the model species. Gutierrez et al. (1997) also obtained a repellent effect from farnesol in the choice bioassay on *M. persicae*. Dancewicz et al. (2010) also studied the effect of farnesol and some of its structural derivatives in a two-choice bioassay similar to ours on *M. persicae*. A repellence against aphids is generated by the aphid alarm pheromone *(E)*-β-farnesene, which alerts them about a danger, but also alerts natural enemies about the aphid's presence (Micha and Wyss 1996). *(E)*-β-farnesene is a compound structurally related to farnesol and both belong to the same farnesene family (sesquiterpenes) (Tesh et al. 1992). Many papers have studied the effect of *(E)*-β-farnesene on aphids, but few have studied farnesol. In 4-arm olfactometry tests with winged adults of *M. persicae*, repellence was obtained with a 7 µl treatment of synthetic *(E)*-β-farnesene (Bruce et al. 2005). Micha and Wyss (1996) also report repellent effects from *(E)*-β-farnesene against *Sitobion avenae* in Y-tube olfactometer bioassays at a dose of 5.7 µg. Our results show a clear repellency by farnesol, like the repellent effect generated by *(E)*-β-farnesene and reported in the literature.

There are not many studies on the effect of EOs on natural enemies. In this work, the EO of aniseed and *(E)*-anethole repels *S. rueppellii*, while citral, *(Z)*-jasmone and methyl salicylate repelled *A. colemani*. Until now, it was unclear whether the larva species from the genus *Sphaerophoria*, possessed organs specialized for detecting odours (Rotheray and Gilbert 2011), but now, with our research, this has been confirmed: syrphid larvae do indeed have olfactory abilities. This is a significant finding which may stimulate further research.

On the other hand, farnesol attracts *A. colemani* and *S. rueppellii* in our olfactometer bioassays. Ma et al. (2017), in two-way olfactometer tests obtained an attractive effect for farnesol on the mirid *Campylomma chinensis* Schuch (Hemiptera: Miridae), an omnivorous generalist predator that can prey on aphids. Other authors studied the effect of *(E)*-β-farnesene on parasitoids (Micha and Wyss 1996; Heuskin et al. 2012; Zhang et al. 2020) and predators of aphids (Francis et al. 2004). In these works, *(E)*-β-farnesene acts as an attractant for natural enemies. Again, our results show that farnesol acts in a similar way to *(E)*-β-farnesene.

In all olfactometer tests, farnesol loses its repellent or attractive effect when it is mixed with *(E)*-anethole. These findings agree with those described by Dawson et al. (1984) who observed that hop plants (*Humulus lupulus*) were readily colonized by aphids in spite of the fact that its EO contains *(E)*-β-farnesene as a major component. They concluded that the presence of the sesquiterpene (−)-β-caryophyllene in the hop EO was responsible for the inhibition of the effect of *(E)*-β-farnesene. In our case, *(E)*-anethole may act in a similar manner inhibiting the effect of farnesol.

We have found that the aphid response to certain EOs and pure compounds is species-specific and even clone-specific. For example, lavandin and fennel only repel *M. persicae* while caryophyllene is repellent only for *M. euphorbiae*. Whereas on *M. persicae*, clone 2 is repelled by lavandin EO and clone 1 by fennel EO. In terms of the activity of the products or the repellent doses, this suggests that there might be morphological or physiological differences in the insect receptors that might explain the differences. Mezei et al. (2020) obtained significant differences when comparing the susceptibility of 13 field populations of *M. persicae* against imidacloprid treatments in a laboratory bioassay. Other authors also reported differences in the responses between clones of *M. persicae* and *A. pisum* to the same treatment (Guilbaurd and Khudr 2020; Yan et al. 2020).

In the contact toxicity bioassay performed on aphids, the most effective treatments were farnesol and its mixture with *(E)*-anethole (1:1). Blending both compounds increased the activity in short treatments times, synergistic effects were not detected. Gutierrez et al. (1997) also observed mortality of *M. persicae* treated with farnesol in residual toxicity bioassays. Bruce et al. (2003) obtained a reduction of the intrinsic ratio of population growth of the aphid *Sitobion avenae* by treating wheat plants previously with *(Z)*-jasmone. In our case, the treatment with *(Z)*-jasmone also reduced the growth rate of *M. persicae* with respect to the control, being negative during the first 72 h due to the mortality caused.

In summary, the results presented in this work show that EOs and certain pure compounds may be of interest as part of integrated control strategies to manage aphid populations in Mediterranean crops. Anyway, further efforts to improve formulations and the application method are still required, especially when these control strategies are to be implemented under field conditions.

Authors' contributions

MCT, PG, MJPV, VFO, JLC, MAMG contributed to conceptualization and methodology. MCT performed experiment. MCT and PG analyzed the data. MCT wrote original draft preparation. All authors contributed critically to the drafts and gave final approval for publication.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability All software used is licensed.

Declarations

Conflict of interests The authors have no conflicts of interests to declare.

Human and animal rights This article does not contain any studies with human participants or animals (other than insects) performed by any of the authors.

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