Research Article

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# Aphicidal activity of farnesol against the green peach aphid – *Myzus persicae*

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## Abstract

**BACKGROUND: *Myzus persicae* (Hemiptera: Aphididae) is considered one of most important agricultural pests in the world. It is one of the main pests in protected pepper crops under glasshouse conditions in Southeastern Spain, but its control is limited as a consequence of the few available authorized insecticides and their incompatibility with the natural enemies. Some essential oils and pure compounds such as anise (*Pimpinella anisum*) or farnesol are repellent and/or toxic to aphids. Their use as a botanical insecticides can be an alternative for aphid control in pepper.**

**RESULTS: The effect of farnesol was evaluated against *M. persicae* in a new bioassay developed to test the contact effect (aqueous formulation of the products) on aphids in laboratory conditions. Aniseed essential oil, geraniol and *(Z)*-jasmone at 0.6% causes an aphid mortality of >50%; and farnesol was the most effective (93.67% mortality). Farnesol nanoemulsions between 0.2% and 0.6% were formulated with an IKA-Labor Pilot dispersing machine (7940 rpm for 10 min) using Tween 80 as a surfactant. These formulations were tested on field experiments (glasshouse conditions) on pepper crops for 2 years. Foliar applications of farnesol at a concentration of 0.4% in field conditions causes a high reduction in aphid populations, with efficacies of ≈70–80% with respect to the control, similar to or even higher than the efficacy of the reference pyrethrin insecticide.**

**CONCLUSION: Farnesol showed a great aphicidal effect against *M. persicae*. The use of this molecule in integrated pest management programs combined with natural enemies is a good option for future control of *M. persicae*.**


**Keywords:** *capsicum annuum*; botanical insecticide; aphids; farnesol; essential oils; integrated pest management

## 1 INTRODUCTION

*Myzus persicae* (Sulzer) (Hemiptera: Aphididae) is considered one of the 15 aphid species of most agricultural importance in the world. It is cosmopolitan, polyphagous and a greatly efficient virus vector (persistent and nonpersistent viruses).[1] *Myzus persicae* has developed resistance to different insecticide groups (carbamates, nicotine, neonicotinoids, organochlorines, organophosphates, pyrethroids and benzoylphenyl ureas) making its control difficult.[2]

This aphid is one of the main pests in protected pepper crops under glasshouse conditions in Southeastern Spain.[3] On pepper crops, aphids are managed with biological control (parasitoids and predators) and chemical treatments.[3–5] The use of chemicals is limited as a consequence of the few available authorized insecticides and uncertain compatibility with natural enemies.[6]

Essential oils (EOs) and their pure compounds are a potential source for the development of botanical insecticides against pests. The literature shows the repellent and/or insecticidal effects of EOs against different insects such as aphids,[7–9] whiteflies,[10] caterpillars[11,12] and stored product pests,[13,14] among others.

In previous works, we studied the repellent and/or insecticidal effects of different EOs and pure compounds on various aphid species: *Rhopalosiphum padi* (L.), *Nasonovia ribisnigri* (Mosley), *Macrosiphum euphorbiae* (Thomas) and *M. persicae*.[15–19] The results of these works show different responses in some cases (repellent and/or aphicidal effect) among these aphid species for the same product.

In this work, we studied the toxic effect of three EOs (aniseed, fennel and coriander) and seven pure compounds [(*E*)-anethole, D-Carvone, citral, farnesol, geraniol, linalool and (*Z*)-jasmone] nanoformulations against *M. persicae* with a contact toxicity laboratory bioassay. *(E)*-anethole is the main compound of aniseed (96.9%) and fennel (30.9%) EOs and linalool (69.9%) of coriander EO. Essential oils and pure compounds were selected based on the repellent effect on *M. persicae* and *M. euphorbiae* determined previously.[18] The aphicidal effect of farnesol nanoemulsions were tested against *M. persicae* in pepper crops grown in pots (two experiments) and soil (three experiments) in glasshouse conditions during 2020 and 2021. The phytotoxic effect of farnesol nanoemulsions on the pepper plants also was assessed in all the experiments.

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## 2 MATERIALS AND METHODS

### 2.1 Insects

*Myzus persicae* (red clone) was collected in Campo de Cartagena (Murcia, Spain) from pepper plants in 2016. The aphids were reared in a growth chamber (25 ± 1 °C, 65% RH and 16 h:8 h, light:dark photoperiod) on pepper plants (*Capsicum annuum* L., cv Herminio; Syngenta US, Greensboro, NC, USA) for several generations. All of the aphids used in the experiments from laboratory cultures were female adults.

### 2.2 Essential oils and pure compounds nanoemulsions

Aniseed (*Pimpinella anisum* L.), fennel (*Foeniculum vulgare* Miller) and coriander (*Coriandrum sativum* L.) (Apiaceae) EOs were obtained from Distilleries Muñoz Gálvez S.A. Company (Murcia, Spain). Essential oils compositions were analyzed in a previous work.[15]

Seven pure compounds were tested: *(E)*-anethole 99% (phenylpropanoid), D-Carvone (monoterpenic ketone), citral 95% (monoterpenic aldehyde), farnesol 95% (alcohol), geraniol, linalool (monoterpenic alcohols) and *(Z)*-jasmone. Pure compounds were obtained from Sigma Aldrich (St Louis, MO, USA).

Essential oils and pure compounds were formulated in an oil in water (O/W) nanoemulsion with Tween80™ (Polysorbate 80) as a nonionic surfactant (Panreac, Barcelona, Spain) at a 1:2 ratio (EO: surfactant). The components were emulsified using a laboratory dispersing machine (IKA-Labor Pilot 2000/4; IKA-Werke GmbH, and Co., Staufen, Germany) at 7940 rpm for 10 min.

### 2.3 Contact toxicity bioassay

Aphids were treated using a computer-controlled spraying apparatus (CCSA) (Burkard Manufacturing Co. Ltd., Rickmansworth, UK) operating at 5 psi and 6v (2 μL cm$^{-2}$) (equivalent application of 200 L ha$^{-1}$). Essential oils and pure compounds were applied at a concentration of 0.6% (v/v) (0.6% EO+1.2% Tween80™) for the initial screening. Groups of five Petri dishes (9 cm$^2$ diameter) were replicated three times (on different days) for each concentration and each product. Aphids (20 wingless female adults) were released on pepper leaf discs (9 cm$^2$ diameter) placed on agar at 1% (w/v) before the treatments. Tween80™ at a concentration of 1.2% (with the same number of aphids used for each product) was used as the control. Mortality was assessed with a fine brush at 24 and 48 h.

For the products that resulted in a mortality >70% in the initially screening (only farnesol did), four concentrations ranging from 0.2% to 0.6% (v/v) were selected to calculate lethal concentrations killing 50% and 90% ($LC_{50}$ and $LC_{90}$).

### 2.4 Glasshouse experiments

#### *2.4.1 Glasshouse experiments in pots*

Semi-field experiments were conducted during spring on two consecutive years (2020 and 2021) in a glasshouse at the Instituto Murciano de Investigación y Desarrollo Agrario y Medioambiental (IMIDA) (37° 56′ 18.1″ N, 1° 08′ 01.1″ W) (Murcia, Spain). Plants of the *Capsicum annuum* cultivar Herminio (Syngenta US) were grown in 2.5-L pots filled with a mixture of peat (Klasmann TS3; Klasmann-Deilmann GmbH, Saterland, Germany) and perlite (Projar, S.A. Co., Valencia, Spain) (3:1). Plants were watered twice a week and fertilized with NPK (15-15-15).

In the first experiment (2020), aniseed EO, *(E)*-anethole and farnesol were tested at 0.6% (v/v). In the second experiment (2021), only farnesol at concentrations of 0.4 and 0.6% (v/v) was evaluated. In each experiment, pyrethrins (Pirecris®; Seipasa Co., Valencia, Spain) at 0.4% were used as a reference insecticide, and Tween80™ (at the same concentration as the highest dose of EO nanoemulsions in each experiment), as a control.

Essential oil nanoemulsions were prepared at a concentration of 2% (v/v) and then diluted to the test concentrations. Nanoemulsions were sprayed using a hand sprayer (Polita 7, Matabi; Goizper Group, Gipuzkoa, Spain) at a rate of ~80 mL plant$^{-1}$.

A randomized block design with three replications (10 plants/treatment each) was used. Ten female adults of *M. persicae* were released on each plant one week before the first count. The number of aphids were counted 1 day before the first treatment (D −1), and then at D1, D2, D3 and D6.

#### *2.4.2 Glasshouse experiments in soil*

Three field experiments were conducted in two consecutive years (2020 and 2021) at the Torreblanca experimental station (37° 46′ 36.8″N, 0° 53′ 49.7″W) (Torrepacheco, Murcia, Spain). The *C. annuum* cultivar Herminio (Syngenta US) was used. Pepper plants were cultivated in two 40-m$^2$ glasshouses at a density of 5 plants m$^{-2}$. A randomized block design with two replications was performed (one in each glasshouse). Each experiment included three treatments: pyrethrins as a reference product (Pirecris®, Seipasa Co.) at 0.4%, and two different concentrations of farnesol. Tween80™ was used as a control. Each treatment was evaluated on 32 pepper plants (16 plants in each replication). Farnesol nanoemulsions were evaluated at concentrations of 0.2% (experiments 2 and 3), 0.4% (all experiments) and 0.6% (v/v) (Experiment 1).

Farnesol nanoemulsions were prepared at 2% (v/v) and diluted to the test dose just before the treatment. A backpack sprayer (Super 16, Matabi, Goizper Group) at a rate of 250 mL plant$^{-1}$ was used to treat the plants.

One pepper leaf with aphids (~20 female adults) from a laboratory colony was placed on each plant one week before the first count. The number of aphids were counted one day before the first treatment (D−1), and then D1, D2 and D7 post-treatment. Natural enemies of aphids were observed on the pepper plants before and after the treatments.

### 2.5 Statistical analysis

The data from the contact toxicity bioassay were analyzed using Polo Plus (LeOra Software, Berkeley, CA, USA). The data were subjected to logit analysis corrected with control mortality.

The data from glasshouse experiments were analyzed using R v4.0.5.[20] The data were adjusted to a negative binomial model, using the glm.nb function of the MASS package,[21] where the number of aphids per plant was the variable response, and the treatment and the block, the factors. The significance of the treatment factor was verified by comparing this model with the restricted model (without the treatment factor), using the anova.negbin function of the same package. Pairwise comparisons of estimated marginal means among treatments were made for each experiment date using the EMMEANS package,[22] fitting the *P*-values with Tukey's test.

Efficacies were calculated with respect to the control (efficacy means from different blocks) using the Henderson–Tilton formula[23]:

$$\text{Efficacy}\,(\%) = \left(1 - (T_a \times C_b)/(T_b \times C_a)\right) \times 100.$$

  

where $T_b$ and $T_a$ are the number of insects in the treated group before and after the treatment, and $C_b$ and $C_a$ are the number of insects in the control group before and after the treatment.

# 3 RESULTS

## 3.1 Topical toxicity

The aniseed EO treatment was the most active to aphids (58.35% of mortality) whilst fennel (14.48%) and coriander (7.98%) had no aphicidal effect at a concentration of 0.6% (v/v). The pure compounds geraniol (52.51%) and *(Z)*-jasmone (50.34%) caused a mortality similar to aniseed EO. Farnesol at a concentration of 0.6% (v/v) was the most active product for aphids, with a mortality of 94.07% at 48 h (Fig. 1). The $LC_{50}$ and $LC_{90}$ of farnesol were 0.394 and 0.575%, respectively, at 48 h (Table 1).

**Table 1.** Mortality and lethal concentrations of farnesol nanoemulsions against *M. persicae* at 24 and 48 h on contact toxicity bioassay

| | Mortality (%)* | |
|---|---|---|
| Concentration (%) | 24 h | 48 h |
| 0 | 1.75 | 3.42 |
| 0.2 | 3.33 | 5.33 |
| 0.3 | 21.38 | 39.39 |
| 0.4 | 35.81 | 45.27 |
| 0.6 | 90.33 | 93.67 |
| $LC_{50}$ (95% CI)† | 0.436 (0.398–0.480) | 0.394 (0.346–0.451) |
| $LC_{90}$ (95% CI)† | 0.603 (0.554–0.682) | 0.575 (0.504–0.630) |

*Average of mortality for each concentration ($n$ = 300).
† All calculations made using Polo Plus software with a Logit model.

## 3.2 Glasshouse experiments

### 3.2.1 Glasshouse experiments in pots

Farnesol then was selected for testing in glasshouse experiments, given the aphicidal effect observed on *M. persicae* in the laboratory bioassay. Aniseed EO and its main compound *(E)*-anethole also were selected for the same reason. The products were evaluated under glasshouse conditions on pepper plants (grown in pots) for two years (April 2020 and June 2021).

*3.2.1.1. Experiment 1.* Products were tested at a dose of 0.6% (v/v). Initial populations were between ≈ 40 and 60 aphids per plant, with statistical differences between farnesol and aniseed EO (LR = 11.29, $P$ = 0.024). Farnesol (efficacies >93%) and pyrethrins (efficacies >97%) were the most effective treatments, with statistically significant differences with respect to the control throughout the experiment (Table 2).

Aniseed EO and its main compound *(E)*-anethole also reduced the number of aphids per plant with respect to the control (LR = 167.94, $P$ < 0.001), with efficacies of 62.47% and 70.7%, respectively, at D2 post-treatment.

However, all of the products tested (except pyrethrins) caused phytotoxic effects on pepper plants. Aniseed EO and its main compound *(E)*-anethole were the most phytotoxic treatments on the crop, and the least effective against aphids than farnesol. For this reason, only farnesol was selected for the posterior glasshouse experiments.

*3.2.1.2. Experiment 2.* Initial populations of *M. persicae* were homogeneous, with ≈90–100 aphids per plant (LR = 1.99, $P$ = 0.574) (Table 2). Pyrethrins were the most effective treatment (efficacies >80%). High reductions in the aphid populations treated with farnesol at a concentration of 0.4 and 0.6% (v/v) were produced in comparison with the control at D1 post-treatment (LR = 97.28, $P$ < 0.001).

The farnesol treatment efficacies were similar at both concentrations (≈65%), without significant differences found between them during the experiment. However, no phytotoxic effects were produced at a concentration of 0.4% on the crop, whereas some effects were found with the 0.6% one.

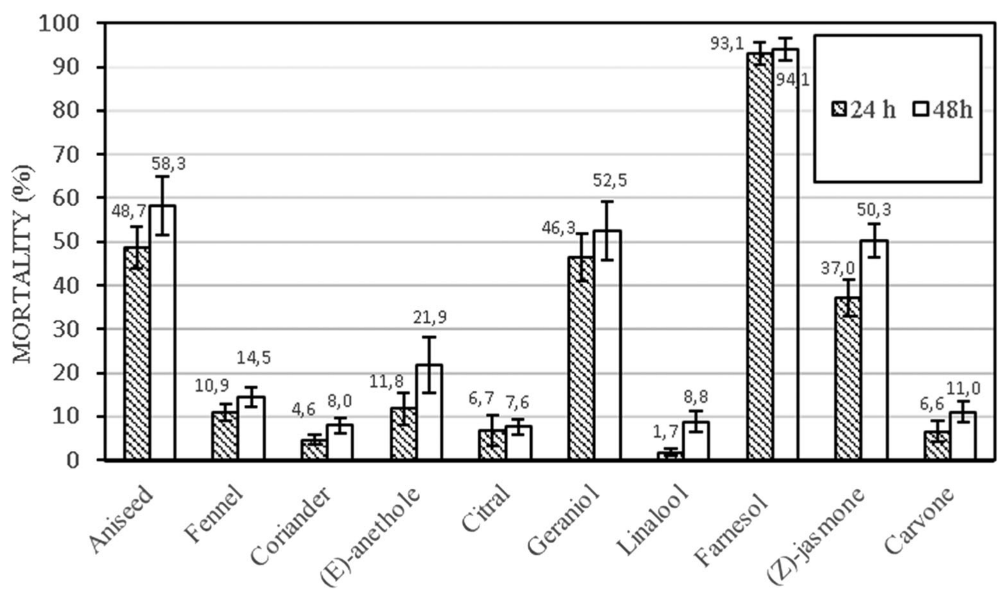


**Figure 1.** Mortality (%) after spraying EO nanoemulsions at 0.6% (v/v) (24 and 48 h at 25 °C) on *M. persicae* Sulzer on contact toxicity bioassay. Insects were tested in three replications (three different days) with five pseudo replications ($n$ = 20 aphids each one) per concentration and formulation ($n$ total = 300 aphids per concentration). Control mortalities were in the range 0.33–7.25% at 24 h and 0.65–11.81% at 48 h.


  

**Table 2.** Aphid populations of *M. persicae* in pepper plants (in pots) before (D−1) and after (D1,2,3,6) spraying with nanoemulsions in glasshouse experiments

| | Treatments | Aphids per plant* | | | | | Efficacy (%)[†] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | D−1 | D1 | D2 | D3 | D6 | D1 | D2 | D3 | D6 |
| Experiment 1 (2020) | (*E*)-anethole 0.6% | 48.13 ± 6.45ab | 31.27 ± 5.22b | 43.77 ± 8.03b | 65.93 ± 9.75b | 175.90 ± 23.55b | 57.96 ± 4.61 | 62.09 ± 2.82 | 60.05 ± 1.61 | 38.09 ± 4.63 |
| | Aniseed 0.6% | 41.23 ± 3.94b | 19.97 ± 2.41b | 29.9 ± 3.12b | 52.13 ± 4.55b | 176.90 ± 14.99b | 68.07 ± 4.91 | 68.71 ± 5.30 | 63.23 ± 2.89 | 28.74 ± 16.16 |
| | Farnesol 0.6% | 66.20 ± 6.72a | 5.57 ± 1.95c | 6.00 ± 1.95c | 3.80 ± 1.26c | 2.67 ± 1.18c | 93.2 ± 4.14 | 95.52 ± 2.27 | 98.29 ± 0.44 | 99.39 ± 0.22 |
| | Pyrethrin 0.4% | 45.33 ± 5.07ab | 1.67 ± 0.43d | 0.70 ± 0.31d | 0.80 ± 0.38d | 2.03 ± 1.01c | 97.41 ± 0.95 | 99.34 ± 0.08 | 99.48 ± 0.12 | 99.23 ± 0.24 |
| | Control[‡] | 61.53 ± 7.97ab | 98.97 ± 14.29a | 149.07 ± 19.34a | 210.97 ± 20.87a | 349.33 ± 12.60a | — | — | — | — |
| | LR/P[§] | 11.29/0.024 | 138.8/<0.001 | 167.94/<0.001 | 178.86/<0.001 | 26 792/<0.001 | | | | |
| Experiment 2 (2021) | Farnesol 0.4% | 91.67 ± 5.74a | 43.00 ± 6.15b | 62.07 ± 7.19b | 80.87 ± 8.10b | — | 66.96 ± 7.11 | 65.12 ± 5.24 | 61.33 ± 4.91 | — |
| | Farnesol 0.6% | 93.77 ± 4.64a | 47.03 ± 5.33b | 64.30 ± 6.69b | 84.37 ± 10.73b | — | 65.71 ± 4.95 | 64.98 ± 4.63 | 61.15 ± 7.00 | — |
| | Pyrethrin 0.4% | 96.27 ± 6.23a | 26.00 ± 3.84c | 32.17 ± 4.89c | 38.20 ± 4.62c | — | 81.48 ± 1.05 | 83.17 ± 1.00 | 82.91 ± 0.51 | — |
| | Control[‡] | 102.30 ± 4.37a | 148.97 ± 6.40a | 202.13 ± 10.76a | 238.07 ± 10.99a | — | — | — | — | — |
| | LR/P[§] | 1.99/0.528 | 97.28/<0.001 | 109.53/<0.001 | 113.87/<0.001 | | | | | |

*Means (± SE) of aphids per plant followed by different letters within the column indicate significant differences among the treatments. Data were analyzed using R software with GLM negative binomial model, calculated with function glm.nb (package MASS) ($n = 30$), and differences between treatments were separated by Tukey's test of Estimated marginal means (emmeans-package).
[†] Efficacy calculated against the control using the Henderson and Tilton (1955) formula.
[‡] Tween80™ 1.2%.
[§] Likelihood ratio statistic (LR) and probability (*P*) by $\chi^2$.

*3.2.2 Glasshouse experiments in soil*

Farnesol nanoformulations at concentrations of 0.2, 0.4 and 0.6% (v/v) were tested under glasshouse conditions on pepper plants grown on soil for two years (in July 2020 and in April 2021).

*3.2.2.1. Experiment 3.* The initial aphid populations were high (>140 aphids per plant) without statistical differences between them (LR = 4.56, $P = 0.207$) (Table 3). Farnesol treatments caused a significant reduction on aphid populations, with statistical differences found with respect to the control during the experiment ($P < 0.001$) (Table 3).

Two days after the treatments, no statistical differences were found between farnesol concentrations, with similar efficacies (≈65%) obtained. Pyrethrins were the most effective treatment, with efficacies ≈80% throughout the entire experiment (Table 3).

Natural enemies of aphids were observed on the pepper plants before and after the treatments. Mummies of parasitoids (1.06–2.88 mummies per plant) and *Aphidoletes* sp. (Diptera: Cecidomyiidae) larvae (1.13–4.13 larvae per plant) were present in every pepper plant at D2 post-treatments. Farnesol at a concentration of 0.6% (v/v) did produce phytotoxic effects on leaves, flowers and fruits. However, no phytotoxic effects were produced by farnesol at a concentration of 0.4%, except for a slight deformation in some leaves of the apical shoot.

*3.2.2.2. Experiment 4.* The initial populations were high (≈250–340 aphids per plant), with significant differences between them (LR = 463.30, $P < 0.01$), and with the highest populations found for farnesol (≈340 aphids per plant). At D2 post-treatments, the aphid populations were reduced with respect to the initial populations, with no significant differences between the final populations. The farnesol treatment at concentration of 0.4% (v/v) reduced initial populations by more than half (122 aphids per plant) at D2 post-treatment, with a greater efficacy than the pyrethrin treatment (efficacies of 69.97 and 50.30%, respectively) (Table 3).

Larvae of *Aphidoletes* sp. were found on the pepper plants before and after the treatments. At D2 post-treatments, larvae were present in all the pepper plants (between 0.25 and 0.88 larvae). No phytotoxic effects were produced by farnesol treatments on the crop, except for a slight deformation found in some leaves of the apical shoot of plants treated at a concentration of 0.4%.

*3.2.2.3. Experiment 5.* Before the treatments, the aphid populations were homogeneous (≈50 aphids per plant) (LR = 1.26, $P = 0.739$) (Table 3). Treatments reduced the aphid populations with respect to the control during the experiment ($P < 0.001$). Pyrethrins and farnesol, at a concentration of 0.4% (v/v), were the most effective treatments (efficacies of >90 and >70% respectively). Farnesol at 0.2% (v/v) reduced the initial aphid populations (44 aphids per plant) to half (23 aphids per plant) two days after the treatment (efficacy >40%) (Table 3).

No phytotoxic effects were produced on the crop with the use of the farnesol nanoemulsion at concentrations of 0.2 and 0.4%.

## 4 DISCUSSION

The bioassay developed in this work assessed the aphicidal effect of products on aphids upon contact. A reference standardized bioassay to study the effect of contact products, such as pyrethroids, is the described by the Action Committee against

**Table 3.** Aphid populations of *Myzus persicae* in pepper plants (in soil) before (D–1) and after (D1,2,7) spraying with nanoemulsions in glasshouse experiments

| | | Aphids per plant* | | | | Efficacy (%)† | | |
|---|---|---|---|---|---|---|---|---|
| | Treatments | D–1 | D1 | D2 | D7 | D1 | D2 | D7 |
| Experiment 3 (2020) | Farnesol 0.4% | 149.00 ± 28.10a | 38.50 ± 8.10bc | 29.50 ± 6.44b | 43.19 ± 10.53b | 65.71 ± 3.35 | 69.48 ± 4.15 | 72.75 ± 1.29 |
| | Farnesol 0.6% | 283.56 ± 30.54a | 74.19 ± 21.31ab | 50.06 ± 12.97ab | 90.56 ± 30.45ab | 66.48 ± 13.56 | 72.92 ± 6.43 | 72.31 ± 4.73 |
| | Pyrethrin 0.4% | 141.06 ± 29.86a | 23.81 ± 6.81c | 17.31 ± 6.55b | 26.19 ± 7.59b | 78.44 ± 6.09 | 80.75 ± 2.00 | 77.84 ± 11.86 |
| | Control‡ % | 180.69 ± 62.72a | 138.38 ± 34.54a | 114.94 ± 29.96a | 192.06 ± 65.13a | — | — | — |
| | LR/*P* | 4.56/0.207 | 19.85/<0.001 | 22.44/<0.001 | 15.66/0.001 | | | |
| Experiment 4 (2020) | Farnesol 0.2% | 342.50 ± 74.65a | 215.25 ± 45.68a | 237.69 ± 49.42ab | 422.06 ± 81.62a | 34.04 ± 4.65 | 46.19 ± 0.50 | 40.68 ± 0.38 |
| | Farnesol 0.4% | 341.25 ± 68.96a | 133.38 ± 37.37a | 122.31 ± 36.92a | 229.44 ± 82.04a | 56.10 ± 13.70 | 69.97 ± 10.26 | 58.19 ± 27.63 |
| | Pyrethrin 0.4% | 232.19 ± 42.61c | 100.69 ± 24.78a | 141.75 ± 37.66a | 295.88 ± 82.97a | 53.22 ± 7.16 | 50.30 ± 16.02 | 31.57 ± 23.70 |
| | Control§ | 282.31 ± 62.29b | 260.63 ± 65.04a | 351.25 ± 90.98a | 541.00 ± 110.21a | — | — | — |
| | LR/*P* | 463.30/<0.001 | 4.85/0.183 | 6.19/0.103 | 4.11/0.249 | | | |
| Experiment 5 (2021) | Farnesol 0.2% | 44.31 ± 4.30a | 23.88 ± 5.68b | 23.63 ± 7.22b | 33.31 ± 11.53b | 41.11 ± 18.92 | 42.38 ± 17.85 | 56.37 ± 3.71 |
| | Farnesol 0.4% | 48.44 ± 3.49a | 11.00 ± 2.13c | 9.56 ± 2.05c | 22.31 ± 6.48bc | 75.98 ± 0.21 | 78.99 ± 1.32 | 73.73 ± 3.65 |
| | Pyrethrin 0.4% | 48.75 ± 4.43a | 3.06 ± 0.77d | 1.25 ± 0.48d | 2.06 ± 0.92c | 93.35 ± 1.10 | 97.24 ± 1.43 | 96.48 ± 2.87 |
| | Control§ | 50.44 ± 2.87a | 47.75 ± 5.09a | 47.56 ± 5.40a | 84.56 ± 16.76a | — | — | — |
| | LR/*P*¶ | 1.26/0.739 | 57.81/<0.001 | 67.99/<0.001 | 45.88/<0.001 | | | |

*Efficacy calculated against the control using the Henderson and Tilton (1955) formula.
† Tween80™ 1.2%.
‡ Tween80™ 0.8%.
§ Likelihood ratio statistic (LR) and probability (*P*) by $\chi^2$.
¶ Means (± SE) of aphids per plant followed by different letters within the column indicate significant differences among the treatments. Data were analyzed using R software with GLM negative binomial model, calculated with function glm.nb (package MASS) ($n = 32$), and differences between treatments were separated by Tukey's test of estimated marginal means (R/EMMEANS).

Insecticide Resistance (IRAC no.019),[24] however, it assesses only the residual toxicity. The bioassay designed in this work assessed the contact *and* residual aphicidal potential of products such as EOs. As a consequence of the high volatility of EOs, a residual toxicity bioassay cannot show the real mortality produced by the product. Therefore, to study the topical effect of EOs or natural products such as pyrethrins, an adequate toxicity bioassay must be taken into account to obtain the optimal concentration of the product.

The results showed a great aphicidal effect of the sesquiterpenoid farnesol against the green peach aphid *M. persicae*. However, the application of this pure compound was difficult owing to its insolubility in water,[25] and its phytotoxicity on lettuce and pepper crops.[19, 26] Aniseed EO and its main compound *(E)*-anethole at 0.6% (v/v) were active against *M. persicae* but produced phytotoxic effects on pepper plants as well. Digilio *et al*.[27] also observed the phytotoxic effect of the vapors of anise and fennel EOs on pepper plants. By contrast, other works showed the insecticidal effect of anise EO and its main compound *(E)*-anethole on other aphid species using similar concentrations (0.2–0.55% v/v), without negative effects on lettuce or cabbage plants.[12,19]

The literature shows the antifeedant and/or aphicidal effect of farnesol against *Myzus persicae*, *Nasonovia ribisnigri*, *Macrosiphum euphorbiae* and *Aphis* fabae.[18,26,28–30] However, the application of farnesol in these studies was carried out under laboratory conditions, and in the present work, we provide new results obtained from the crop under real-world cultivation conditions. In this work, we tested aqueous nanoformulations of farnesol compatible with pepper cultivation. The application of farnesol nanoemulsions at 0.4% (v/v) produced high mortalities in aphid populations, without causing damage to the crop. However, a better efficacy of the treatments (>70%) was obtained when the aphid populations were lower (glasshouse experiments 1 and 5 with ≈50 aphids per plant before the treatments), in comparison with other experiments (>100 aphids per plant).

Farnesol has multiple uses, such as in the perfume or food industries.[31,32] In insects, it was discovered as a juvenile hormone of the mealworm Tenebrio.[33] It also is known as a mite pheromone,[34] ant and aphid repellent[18,35] or attractant of bees.[36] Also, the attractant effect of farnesol on the syrphid *S. rueppellii*, the parasitoids *Aphidius colemani* Viereck and *A. gifuensis* (Ashmead) (Hymenoptera: Braconidae), and the mirid *Campylomma chinensis* (Schuch) (Hemiptera: Miridae) was demonstrated in two-way olfactometer experiments.[18,37,38] Applications of farnesol are compatible with some aphid's natural enemies. In a previous work, Cantó-Tejero *et al*.[18] did not observe negative adverse effects when larvae of the hoverfly *Sphaerophoria rueppellii* (Wiedemann) (Diptera: Syrphidae) were exposed to residual treatments of farnesol nanoemulsions at 0.3% (v/v) in laboratory conditions (mortality of 2.5%). Also, cecidomyiid larvae (aphid predators) were observed in plants treated with farnesol during glasshouse experiments. The use of farnesol in integrated pest management (IPM) programs is a good option, as it is compatible with some natural enemies of aphids. In this sense, this product could be included on an IPM program using a push–pull strategy[39] (spraying aphid colonies causes mortality and helps to establish parasitoid populations in the glasshouse).

Farnesol belongs to the farnesene family, such as *(E)-β*-farnesene, and they are structurally similar. In fact, farnesol can be used to synthesize *(E)-β*-farnesene.[40] Cantó-Tejero *et al*.[18] concluded that farnesol acted in a similar manner as the aphid's alarm pheromone, *(E)-β*-farnesene (repellent to aphids and attractant to parasitoids). Many authors report the attractant effect exerted by *(E)-β*-farnesene on parasitoids[41,42] and predators[43] of aphids. Gut and Van Oosten[44] indicated that *(E)-β*-farnesene exerted a toxic effect when it was applied topically on aphids. Again, these results show a similar effect of farnesol as the alarm pheromone when it was applied on aphids.

Farnesol is present as the main compound in different EOs of flowers[45–47] or stems and leaves[48–50] of plants of different botanical families. Nanoformulations of farnesol or EOs rich in this sesquiterpenoid are a good option for the control of aphids. Current prices of pyrethrum dry flowers (with 50% pyrethrin content) before processing are expensive (€175 $kg^{-1}$).[51] The price of 25 g farnesol (€45, CAS no. 4602-84-0, Sigma-Aldrich) is less than half the same mass of pyrethrum extract (with 50% pyrethrin content) (€105, CAS no. 8003-34-7, Sigma-Aldrich).

The formulations presented in this work showed a great aphicidal effect (similar to the reference insecticide). When EOs are formulated as nanoemulsions, the particle size is lower, increasing their solubility and biological activity.[16] In previous works, different farnesol nanoemulsions, using Tween80™ as a surfactant at a 1:2 ratio (prepared using ultrasound or high speed rotor) at different concentrations, were characterized. Farnesol nanoemulsions were formulated at 1 and 2% (v/v) and then diluted to 0.25%. These had particle sizes of 100 nm, Z-potential of −15 mv and a polydispersity index (PDI) between 0.1 and 0.5[15,16] (unpublished data). In fact, in the aforementioned works, farnesol formulations at the same concentrations, but using soy lecithin as the surfactant, also were characterized, resulting in larger particle sizes (150–500 nm), PDI (0.4–0.6) and greater stability, with *Z*-potential values between −40 and − 50 mV.

The mode of action (MoA) of farnesol on aphids is unclear. It acts as a precursor of the juvenile hormones of holometabolous insects and its absence in the larval stage induces metamorphosis.[52] Knowing the MoA of farnesol would allow for an improved effectiveness in the formulations, because if its activity is the result of a hormonal effect, its application should be performed during the nymphal stages of aphids. This could explain why it is more effective when applied to smaller colonies (with a greater number of nymphs).

These results are promising, yet the farnesol nanoemulsions used in this work need to be improved. The physical properties of these nanoemulsions were not adequate because during their application on pepper plants, drops of the treatments had a lower surface tension, coalescing into larger drops as compared to the reference pyrethrin-based insecticide. This may be one of the causes of the phytotoxicity (apart from concentration). Surfactants are a fundamental part in the formulation, and their proper selection is essential to obtain a stable and effective nanoemulsion.[53] As mentioned previously, in prevoius studies, we verified how the use of different surfactants influenced the different characterization parameters of nanoemulsions. Further research is needed to obtain an aphicidal product at concentrations of <0.4% and compatible with the pepper crop.

## AUTHOR CONTRIBUTIONS

MC-T: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing-Original Draft, Writing-Review & Editing, Visualization; PG: Conceptualization, Methodology, Software, Formal analysis, Writing-Review & Editing, Visualization; MJP-V: Conceptualization, Methodology, Writing-Review & Editing, Visualization, Funding acquisition. All authors contributed critically to the drafts and gave final approval for publication.

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## CONFLICT OF INTERESTS

The authors have no conflicts of interests to declare. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

## DATA ACCESSIBILITY

The data of this study are available from the corresponding author on reasonable request.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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