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Impact of farnesol nanoformulation on the movement of melon aphid and the spread of aphid-transmitted viruses

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Abstract

The management against *Aphis gossypii* Glover (Hemiptera: Aphididae) and aphid-transmitted cucumber mosaic virus (CMV, *Cucumovirus*) and cucurbit aphid-borne yellows virus (CABYV, *Polerovirus*) relies on synthetic pesticides and natural enemies, which can limit the spread of persistently-transmitted viruses but enhance non-persistent viral transmission. Nanoemulsions of biopesticides can help prevent insect resistance and shortage of authorized active ingredients. They can also solve problems associated with the formulation of natural substances. The phytotoxicity of nanoformulations of farnesol and aniseed was evaluated on melon plants. Farnesol caused brown circular sports and malformations on treated leaves that disappeared 2–4 weeks after application. The settlement and movement of *A. gossypii* were studied with EthoVision video tracking. The application of natural actives altered aphid behavior, increasing their movement and reducing the time they spent on plants. The primary and secondary spread of CMV and CABYV was evaluated under greenhouse conditions. Pesticide application before aphid release did not prevent the primary spread of non-circulative CMV, but reduced the spread of persistently-transmitted CABYV. Farnesol nanoemulsion is an encouraing option but its activity might be reinforced with the introduction of natural enemies. Our findings highlight that the application of respectful alternatives must be accompanied with an extensive insight of their individual biological features and impacts toward targeted pests and host plants.

Keywords CMV · CABYV · Aniseed · Virus transmission · Phytotoxicity · Ethovision

Key message

- The current control strategies of aphid *Aphis gossypii* enhance non-persistent viral transmission.
- Nanoformulation of biopesticides can improve their biological activity and persistence.
- Farnesol nanoformulation causes phytotoxic symptoms that disappeared 2–4 weeks after application.
- Aphids walk more distance while remaining less time settled on farnesol-treated disk.
- Farnesol application before aphid release reduces the spread of persistently-transmitted CABYV.

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Introduction

Melon (*Cucumis melo* L.) is a cucurbit crop originated from South-east Asia, domesticated and imported to Europe more than 3,000 years ago (Walters 1989). The organoleptic properties of its fruits have placed it as one of the most important cultivated species, with a global area reaching 1 million hectares and a total production of more than 28 million tons (FAOSTAT 2024). Melon crop can be impacted by a variety of insect pests and diseases that reduce its yield

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and cause important economic losses (Strange and Scott 2005; Hull 2014).

The cotton aphid *Aphis gossypii* Glover (Hemiptera: Aphididae) is a cosmopolitan species with more than 600 host plants. This aphid is able to transmit more than 50 plant viruses (Kennedy et al. 1962). Among these, cucumber mosaic virus (CMV, *Cucumovirus*) and cucurbit aphid-borne yellows virus (CABYV, *Polerovirus*) are two of the most prevalent viruses on field and protected cucurbits in the Mediterranean basin at the present time (Moya-Ruiz et al. 2023; López-Martín et al. 2024). These viruses differ on the site in which the viral particles are retained by the aphid vector and the retention period. CMV is non-circulative, non-persistently-transmitted virus, with short acquisition and inoculation periods lasting seconds, without latency, retained in the stylet cuticle of the aphid (Hull 2014; Ng and Perry 2004). CABYV is circulative, persistently-transmitted virus, with long acquisition and inoculation periods lasting several hours, and it relocates from the midgut and hindgut into the salivary glands before inoculation (Hull 2014).

The management against *A. gossypii* and aphid-transmitted viruses is primarily based on biological control with parasitic wasps and predators, with occasional chemical treatments to control outbreaks (van der Blom 2017). The use of synthetic pesticides and natural enemies can limit the spread of persistently-transmitted viruses but has the risk of enhancing non-persistent viral transmission (Jeger et al. 2011, 2012; Dáder et al. 2012; Raccah and Fereres 2009).

Aphids have developed resistance to many synthetic pesticides (Simon and Peccoud 2018; Wang et al. 2022), and EU policy aims to reduce the number of pesticides available in the near future. Therefore, there is an urgent need to search for novel natural alternatives, as the number of synthetic active ingredients authorized for insect control under European legislation is decreasing (EU 2024). Biopesticides include naturally occurring substances (biochemical pesticides), microorganisms (microbial pesticides), and substances produced by plants containing added genetic material (plant-incorporated protectants) (EPA 2024). They are naturally less toxic to the environment and have narrow spectrum of activity. However, natural substances used in plant protection often have high volatility, resulting in low persistence. Additionally, their non-polar nature necessitates the use of solvents and emulsifiers to form stable emulsions (Isman 2006; Tariq et al. 2019; Valdivieso-Ugarte et al. 2019; López et al. 2021). The nanoformulation of these biopesticides, by ultrasound or high-speed rotor, allows to obtain spontaneous colloidal emulsifications (Anton and Vandamme 2011). This increases the water solubility and interface area between the components, and thus the penetration, biological activity, and persistence of the active ingredient (Klang and Valenta 2011; Shah et al. 2016; Pascual-Villalobos et al. 2017, 2019, 2022).

In this research, we studied aniseed (*Pimpinella anisum* L.), which includes the aromatic ether phenylpropanoid (E)-anethole [*trans*-1-methoxy-4-(C1-propenyl) benzene] in its chemical composition, and farnesol, a natural sesquiterpene alcohol (Addae-Mensah et al. 1996; Blewitt and Southwell 2000), as active compounds against *A. gossypii* and virus transmission. Both compounds exhibit protection against different aphid species in laboratory and greenhouse conditions (Pascual-Villalobos et al. 2017; Isman 2020; López et al. 2021). As an example, settlement and olfactory assays showed how farnesol and aniseed repelled *Rhopalosiphum padi* L., *Macrosiphum euphorbiae* Thomas and *Myzus persicae* Sulzer (Hemiptera: Aphididae) (Pascual-Villalobos et al. 2015, 2017, 2019; Cantó-Tejero et al. 2022a). Farnesol and (E)-anethole can be mixed together to reduce populations of *M. persicae* and *M. euphorbiae* in sweet pepper plants after foliar application (Cantó-Tejero et al. 2022a).

So far, research on nanoemulsions and aphids has been limited to toxicity, repellence and feeding behavior assays. The settlement behavior of aphids and virus transmission has not been studied previously. It is preceptive to characterize the biological features of nanoformulations that could enhance the ecological control approach in a melon crop. Therefore, in this work, we present novel information on the settlement and movement of *A. gossypii*, and the primary and secondary spread of CMV and CABYV on melon plants treated with farnesol nanoemulsion under greenhouse conditions. Both CMV and CABYV produce high yield losses in cucurbit crops in melon (Moya-Ruiz et al. 2023; López-Martín et al. 2024). Moreover, the potential phytotoxicity of nanoformulations of farnesol and aniseed on melon plants was evaluated.

Materials and methods

Melon plants

Seeds (*C. melo* cv. Bazan; Fitó, Barcelona, Spain) were germinated on wet filter paper inside Petri dishes (12 cm diameter). Seedlings were transplanted to pots filled with vermiculite and soil substrate (1:2; Castillo Arnedo, La Rioja, Spain). The pot was of different size depending on the experiment: 13 cm diameter for phytotoxicity assays, 13 cm diameter for behavior studies and 7 × 7 cm for virus spread tests. Plants were grown inside an insect-proof walk-in chamber (16:8 h L:D photoperiod, 25:20 °C, 65–70% relative humidity (RH)). Plants were watered three times a week using a NPK solution (20–20-20; Nutrichem 60 fertilizer, Miller Chemical & Fertilizer Corp., Pennsylvania, USA) at 1 g L^{-1} dosage.

Aphid rearing

A colony of *A. gossypii* was maintained on melon plants inside a walk-in chamber a climatic chamber (16:8 h L:D photoperiod, 24:20 °C and 65–70% RH). The colony was initiated from a single virginoparous apterae collected on melon in 1998 at El Ejido (Almería, Spain). Alate aphids were collected with an aspirator just before the experiments.

Virus isolates

CMV isolate M06 was obtained from melon in 1996 in Tarragona (Spain) and maintained by mechanical inoculation on melon plants (Díaz et al. 2003). The CABYV isolate was obtained from zucchini squash in 2003 in Montfavet (France) and maintained by aphid serial transmission (Moreno et al. 2011). Briefly, viruliferous *A. gossypii* were reared in CABYV-infected melon plants that were serially inoculated by aphids in advance. To generate CABYV-infected plants 15–20 viruliferous adults and nymphs were transferred to healthy plants (BBCH-scale 12) for an inoculation access period (IAP) of 72 h. After that, aphids were removed. Infected plants were maintained inside an insect-proof walk-in chamber (16:8 L:D photoperiod, 24:20 °C and 65–70% RH). Infection was confirmed by Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) using specific commercial antibodies against CMV (Agdia, Indiana, USA) and CABYV (Sediag, Bretenière, France) 4 weeks after inoculation.

Natural active substances

Two active substances were provided by Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (Murcia, Spain): farnesol (Sigma Aldrich, Merck Life Science, San Louis, Missouri, USA) and aniseed essential oil (EO) (*P. anisum*, Apiaceae) (Destilerías Muñoz Gálvez, Murcia, Spain). Active compounds were formulated at 0.25 and 0.5% concentration (v/v) in distilled water with Tween80 (1:2; Panreac, Barcelona, Spain) or with soybean lecithin and sunflower oil (1:1:1), and stored at 4 °C until use.

Aniseed EO was subjected to analysis by GC–MS, which determined that the main compound was (E)-anethole (96.9%). 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^{-1}, and finally raised to 250 °C at a rate of 10 °C min^{-1}. 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 (Pascual-Villalobos et al. 2017).

Nanoemulsion formulations

Nanoemulsions of both farnesol and aniseed were prepared as oil-in-water formulations using a high-speed dispersion machine IKA Labor-Pilot Controller 2000/4 (IKA-Werke GmbH and Co. Staufen, Germany) programmed to work each batch of 100 mL for 10 min at a rotor speed of 794 rpm and a cooling temperature of 15 °C, which prevents overheating of the machine, maintaining the temperature in the mixing chamber at 20 ± 5 °C and thus preventing losses of the bioactives by volatilisation during the process (Pascual-Villalobos et al. 2022). Nanoemulsions were characterized just before utilization. Particle size, polydispersity index, and Z-potential were measured using a Zetasizer Advance Ultra Red Label (Malvern Panalytical Ltd., Spectris, Madrid, Spain) by multi-angle dynamic light scattering (MADLS). Light scattering by nanoemulsions was detected at 45, 90, and 173° independent angles. The fluctuations of the scattering intensity were then analyzed by the equipment using a technique called autocorrelation. In MADLS, the correlation data from the three detection angles are combined, enabling a more reliable and accurate representation of the particle size distribution. Fresh formulations were used on each experiment. Solutions were readily used after formulation. Storage time since preparation was 1 month.

Phytotoxicity of nanoemulsion formulations on melon plants

Four nanoemulsions were studied: farnesol and aniseed, mixed with either Tween80 or soybean lecithin and sunflower oil. Nanoemulsions were tested at 0.25% and 0.5% concentration (v/v) of active compounds. Three control treatments were included: distilled water, Tween80, and soybean lecithin and sunflower oil, at the corresponding concentrations. Five melon plants (BBCH-scale 12), potted separately in 13 cm diameter pots, per treatment were treated at a rate of 0.6 mL $plant^{-1}$, using an aerograph connected to a pressure pump (0.4 MPa) (FD-186 220 V, Fengda, The Netherlands). Randomized sets of potted plants from the

seven different treatments were covered with an insect-proof fabric and maintained in trays in a randomized disposition inside a greenhouse (natural photoperiod of 12:12 (L:D), 22:18 ± 2 ºC, 65–70% RH) to avoid positional effect. Plants were rotated 180º daily. The evaluation of symptoms started 4 days after the treatment and continued once a week during four weeks. Phytotoxic symptoms were considered by visual evaluation. The chlorophyll content index (CCI) was calculated as the ratio between the optical absorbance in wavebands 653 and 931 nm using a chlorophyllmeter (CCM-200, Opti-Sciences, Hudson, USA) (n = 5 per treatment). To correlate this arbitrary CCI value to quantitative data, fresh leaf material (1 cm^2) was crushed in a mortar and pestle with 2 mL of 80% acetone (v/v) as solvent for chlorophyll extraction (n = 24). Absorbance was measured in wavebands 645 and 663 nm using a spectrophotometer (Optima 4300 DV, Perkin Elmer, Waltham, USA). Total chlorophyll was calculated by Arnon equation (Arnon 1948; Khaleghi et al. 2012). These values were corrected to match the area measured by the chlorophyllmeter. Then, CCI and total chlorophyll data were graphically correlated (Total chlorophyll (mg g^{-1}) = $2e^{-5}$ CCI + $5e^{-6}$; $R^2 = 0.78$)). The slope of the regression was used as the conversion factor to obtain the quantitative data from the CCI values.

Settlement behavior of aphids on treated plants

The effect of farnesol with Tween80 nanoemulsion at 0.5% concentration (v/v) was studied. Aniseed formulation was not included, due to promising farnesol results from experiments that were being carried out simultaneously in the group now published, which reduced aphid probing time on pepper plants (Pascual-Villalobos et al. 2017, 2019; Cantó-Tejero et al. 2022a, b, c; Martín et al. 2024).

Two control treatments were included: distilled water and Tween80 at 0.5%. Two active ingredients of widely-used synthetic insecticides with different mode of action were included as control treatments: acetamiprid (nicotinic acetylcholine receptor competitive modulator; Epik, acetamiprid 20% w/w [SP], Sipcam, Valencia, Spain) and pyrethrin (sodium channel modulator; Pirecris, pyrethrin 2% w/v [EC], Seipasa S.A., Valencia, Spain), both at a rate of 1 L ha^{-1} (IRAC 2024). Melon plants (BBCH-scale 13) were treated sprayed until runoff point. The movement pattern of *A. gossypii* wingless adults on treated leaf disks was studied with EthoVision XT8 integrated video tracking system (Noldus Information Technology, Wageningen, The Netherlands). EthoVision software automatically determines the point-by-point location of the aphid within the experimental arena and calculates parameters derived from changes in position. Following Moreno-Delafuente et al. (2013), leaf disks (2.5 cm diameter) were cut, avoiding pronounced central nerves to prevent interferences with the camera tracking system. Leaf disks were cut 2, 24, and 48 h after treatment application and used within a 2-h interval after excision. One disk was placed showing the abaxial side on the center of a Petri dish (9 cm diameter), fixed with an agar layer (3%). One non-starved aphid was placed on the middle of the disk. Movement was tracked over the experimental arena (63.5 cm^2) with a video camera (WV-CP500/g, Panasonic, Mastsushita Electric Industrial, Osaka, Japan) for 10 min (n = 15–24, depending on treatment). The parameters analyzed were distance walked and timespan on the disk (Noldus et al. 2002; Noldus Information Technology 2009). Aphids were used only once. Recordings in which aphids abandoned the experimental arena were considered invalid. Aphids that moved less than 1 mm during the 10-min recording were considered stationary. Stationary and invalid aphids were discarded from the statistical analysis.

CMV and CABYV primary and secondary spread under semifield conditions

The effect of farnesol with Tween80 nanoemulsion at 0.5% concentration (v/v) was studied on the primary and secondary spread of CMV and CABYV inside an experimental greenhouse under controlled conditions (16:8 L:D photoperiod, 24:18 ± 2 °C and 70–80% RH), adapting the methodology from Dáder et al. (2012). Two control treatments were included: water-mock control and acetamiprid. Insect-proof cages (1 × 1 × 1 m) containing four trays filled with vermiculite and soil substrate (1:2) forming a continuous and uniform surface were used as the experimental arena (n = 3 replicates per treatment). In order to evaluate the primary spread, 49 healthy melon plants (BBCH-scale 12) were placed in a 7 × 7 square disposition with a distance of 12.5 cm between plants (Fig. 1a). Plants were treated depending on the distribution pathway of the active substances. Treatments water-mock control and systemic active ingredient acetamiprid were applied 24 h before aphid release using a hand sprayer (Berry 1.5, Matabi, Goizper, Guipuzkoa, Spain). Farnesol, highly volatile, was applied 2 h before using an aerograph under the same conditions described in the phytotoxicity experiment. To generate CABYV-viruliferous alates, the population was reared on infected melon plants for an acquisition period access (AAP) of 2 weeks before the release. To generate CMV-viruliferous alates, aphids were reared on healthy melon plants until the day of the release. That day, aphids previously starved for one hour, were allowed to feed on a detached CMV-infected melon leaf for an AAP of 5 min before the release. For both cases, 200 viruliferous *A. gossypii* alates were released from black glass tubes placed on a platform hanging at the top of the cage (50 cm distance). Once released, aphids were allowed to freely fly and feed on the healthy plants for an IAP of 24 h for CMV

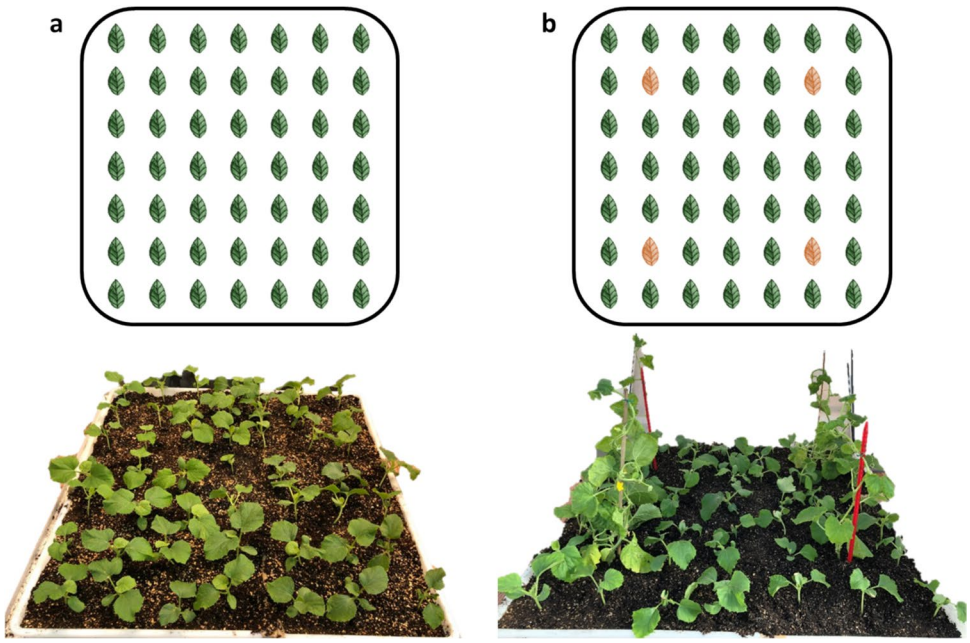

Fig. 1 Spatial distribution scheme of the experimental arena for CABYV and CMV spread experiments. Green leaves symbolize healthy host melon plants, whereas red leaves represent virus-infected source plants. **a** Primary spread. **b** Secondary spread

and 48 h for CABYV. In order to evaluate the secondary spread, 45 healthy melon plants (BBCH-scale 12) and four viral source melon plants (4-weeks post-inoculation) were placed in the same 7 × 7 square disposition (Fig. 1b). Two hundred non-viruliferous aphids were released from the flight platform and allowed to feed on the plants during an IAP of 7 days for CMV and 2 days for CABYV. Cages were rotated 90º daily to avoid orientation bias. For every experiment, once IAP was finished, each melon plant was treated by drench with 15 mL imidacloprid (Confidor, 20% w/v SL; Bayer CropScience, Barcelona, Spain) to kill aphids. Viral infection was confirmed by DAS-ELISA as described previously 4 weeks after inoculation.

Statistical analysis

Data were analyzed using SPSS v. 29.0 (IBM Statistics, Chicago, USA). Count data from the phytotoxicity and aphid behavior experiments were transformed with logistic or angular transformation to decrease heteroscedasticity and achieve normal distribution. Unifactorial analysis of variance (ANOVA, $P \leq 0.05$), followed by Tukey or LSD pairwise comparison test, was used whenever variables followed the premises. Non-parametric Kruskal–Wallis *H* followed by Dunn test was applied if data did not meet the requirements for parametric analysis after the transformation of variables. The parameter "movement" from the aphid behavior experiment was analyzed by Chi-square (χ^2) 2 × 2 contingency table with Yates correction ($P \leq 0.05$). Data from the viral spread experiment were analyzed by χ^2 or Fisher test (if expected values were below 5) ($P \leq 0.05$), using StatView 4.01 for Macintosh (Abacus Concepts, Berkeley, USA).

Results

Characterization of nanoemulsions

Formulations were polydisperse although with main peaks less than 1000 nm in size (Table 1). Z-average was very small, 47 and 42 nm, for the farnesol formulations with Tween80 (1:2). Otherwise values for aniseed EO or farnesol fell within the range 133–407 nm, also nanometric, in both formulations. In general, the use of soybean lecithin and sunflower oil (1:1:1) instead of Tween80 gave larger particles but more stability on account of more negative values of Z-potential (Table 1). Characterization was done just before experimental use. Changes in PDI, particle size or Z-potential were not measured over time. Nanoemulsion storage time was 1 month, probably, there were some changes during this time but still the values measured indicated acceptable parameters.

Phytotoxicity of nanoemulsions on melon plants

Application of farnesol nanoemulsion at both concentrations (0.25 and 0.5%) caused visual symptoms of phytotoxicity, regardless the surfactant, either Tween80 or soy lecithin and sunflower oil. Brown circular spots appeared on fully developed treated leaves 2 days after treatment (Fig. 2b). Some treated leaves, which were not fully developed at the time of application, showed malformations 1 week after treatment (Fig. 2c,d). Malformations on new leaves disappeared 2 weeks after treatment with farnesol at 0.25% and 4 weeks after treatment with farnesol at 0.5%. Treated

Table 1 Characterization of oil-in-water nanoemulsions in two formulations (Tween80 (1:2) or soy lecithin and sunflower oil (1:1:1)) at two concentrations (0.25 and 0.5%)

Product	Concentration (%)	Z-average (nm)	PDI[a]	Peaks (%)[b]	Particle size (nm)	Z-potential (mV)
Tween80 (1:2)						
Aniseed EO	0.5	133 ± 22.745	0.887	73	331	−23.11
				15	18	
				12	3356	
	0.25	197 ± 52.463	0.861	51	248	−20.82
				35	1295	
				10	16	
Farnesol	0.5	47 ± 0.223	0.417	86	86	−18.99
				13	15	
	0.25	42 ± 0.172	0.3	100	63	−13.60
Control[c]	0.5	10 ± 0.044	0.268	88	10	−7.29
				12	1452	
	0.25	121 ± 92.442	0.298	83	9	−6.67
				15	285	
Soy lecithin and sunflower oil (1:1:1)						
Aniseed EO	0.5	371 ± 17.226	0.687	45	555	−31.79
				44	179	
				11	4986	
	0.25	350 ± 10.308	0.676	68	280	−34.20
				18	4652	
				13	1058	
Farnesol	0.5	374 ± 18.851	0.461	58	278	−16.71
				35	1024	
				7	4704	
	0.25	407 ± 5.452	0.592	86	540	−14.50
				14	4367	
Control[c]	0.5	250 ± 8.876	0.533	82	295	−28.15
				18	3550	
	0.25	282 ± 1.531	0.692	76	324	−26.12
				24	4118	

[a]Polydispersity index

[b]Only peaks with > 5% in intensity were included

[c]Control was prepared the same but the natural active substance (aniseed EO or farnesol)

melon plants were shorter but completed their phenological development.

The chlorophyll content of melon plants treated with 0.25% aniseed with Tween80 was significantly lower than on plants treated with the surfactant only after 4 days ($F_{6,32} = 3.54$, $P = 0.008$), 11 days ($F_{6,32} = 3.99$, $P = 0.004$), and 18 days of treatment ($H = 14.35$, $P = 0.026$) (Fig. 3a). No significant differences were observed among treatments after 25 days ($F_{6,33} = 1.74$, $P = 0.142$). The chlorophyll content of plants treated with 0.5% farnesol or aniseed with Tween80 was significantly lower than on plants treated with only the surfactant after 4 days ($H = 12.70$, $P = 0.048$) and 11 days of treatment ($F_{6,33} = 2.48$, $P = 0.043$) (Fig. 3b). No significant differences were observed among treatments after 18 days ($F_{6,33} = 2.22$, $P = 0.066$) and 25 days ($F_{6,33} = 0.13$, $P = 0.99$).

Settlement behavior of aphids

The proportion of aphids that moved on leaf disks after 2 and 24 h of compound application was similar among treatments (Table 2). However, after 48 h of treatment, the proportion of mobile aphids on disks treated with acetamiprid was lower than on pyrethrin ($\chi^2 = 5.00$, $P = 0.025$) and farnesol ($\chi^2 = 4.54$, $P = 0.033$).

The distance walked by aphids 2 h after pesticide application was significantly reduced on leaf disks treated with Tween80 and acetamiprid, compared to pyrethrin and farnesol ($H = 13.43$, $P = 0.009$) (Table 2). When the evaluation was done after 24 h, aphids walked significantly more distance on pyrethrin disks ($H = 16.74$, $P = 0.002$). After 48 h, aphids walked significantly more distance on pyrethrin

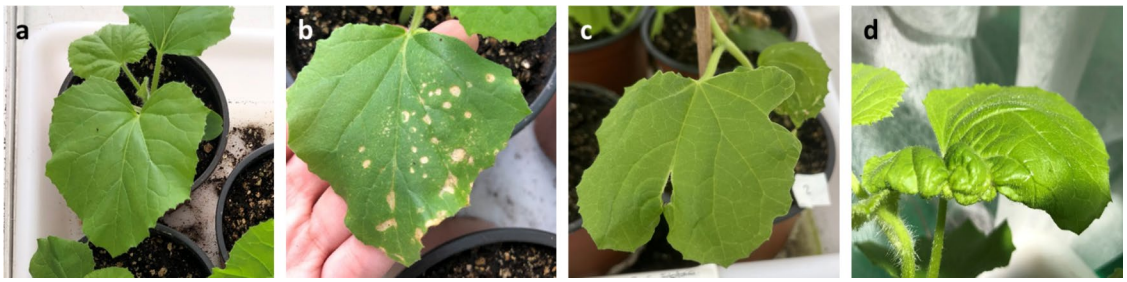

Fig. 2 Phytotoxic symptoms in melon plants. **a** Water-mock control. **b** Brown circular spots 4 days after treatment with farnesol mixed with soy lecithin and sunflower oil (1:1:1) nanoemulsion at 0.25% (v/v). **c** Leaf malformation 21 days after treatment with farnesol mixed with Tween 80 (1:2) nanoemulsion at 0.5% (v/v). d) Leaf malformation 10 days after treatment with farnesol mixed with soy lecithin and sunflower oil (1:1:1) nanoemulsion at 0.5% (v/v)

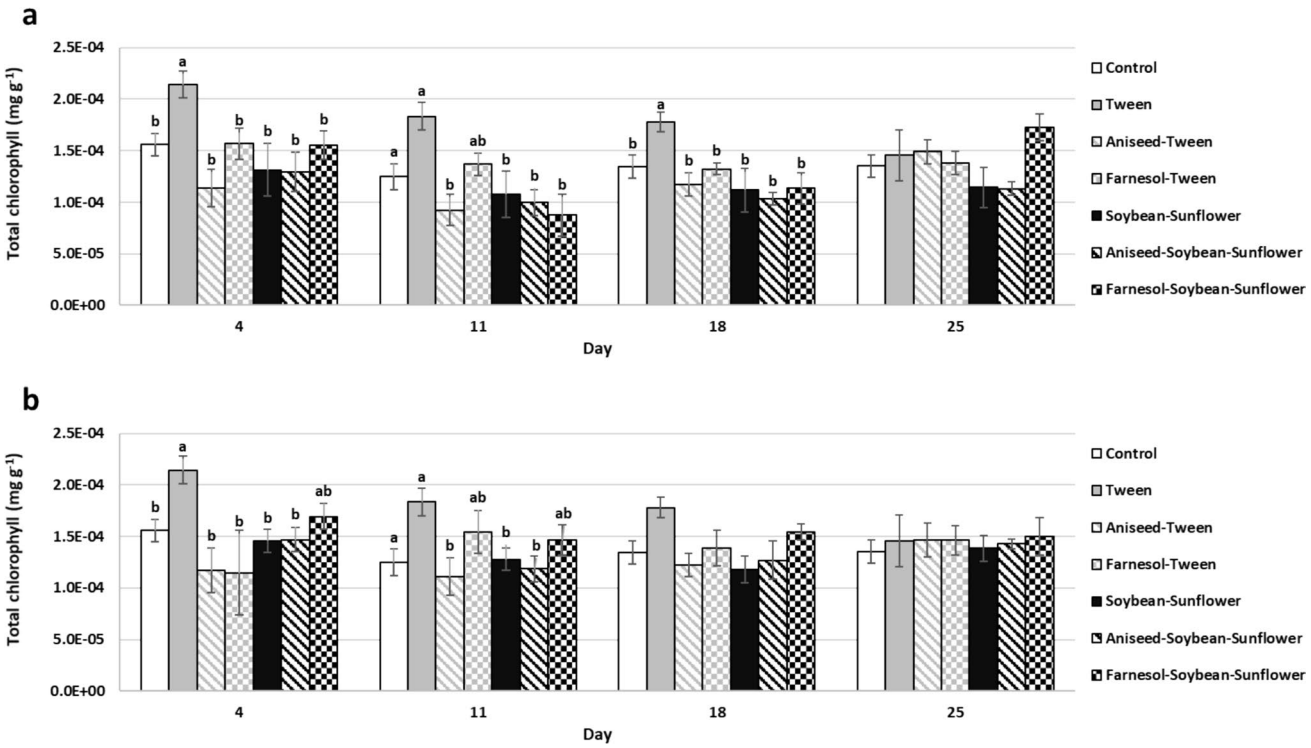

Fig. 3 Mean values ± SEM of total chlorophyll concentration (mg g^{-1}) of melon leaves evaluated 4, 11, 18, and 25 days after treatment application. Control treatments were distilled water, Tween80, and soy lecithin and sunflower oil (1:1). Natural active substances were aniseed and farnesol, mixed with either Tween80 (1:2) or soy lecithin and sunflower oil (1:1:1) nanoemulsions. **a** Solutions at 0.25% (v/v). **b** Solutions at 0.5% (v/v). Different lowercase letters indicate significant differences among treatments by unifactorial ANOVA or Kruskal–Wallis H test ($P \leq 0.05$)

and farnesol disks with regard to water-mock and acetamiprid ($H = 9.56$, $P = 0.048$).

Besides, aphids spent significantly less time on pyrethrin disks after 2 ($H = 12.35$, $P = 0.015$) and 24 h of treatment ($H = 24.82$, $P < 0.001$), and on farnesol disks after 48 h of treatment ($H = 12.69$, $P = 0.013$) (Table 2).

Impact of farnesol formulations on CMV and CABYV spread

The primary spread of CMV was similar among treatments (Table 3). Acetamiprid significantly reduced the secondary spread of CMV compared to water-mock ($\chi^2 = 27.1$, $P < 0.001$) and farnesol ($\chi^2 = 30.0$, $P < 0.001$). Acetamiprid ($\chi^2 = 18.0$, $P < 0.001$) and farnesol ($\chi^2 = 8.4$, $P = 0.004$) significantly reduced the primary spread of

Table 2 Settlement behavior of *Aphis gossypii* on melon leaf disks evaluated 2, 4, and 48 h after treatment application

Evaluation (h)	Treatment	Aphids evaluated	Aphids that moved	Movement (%)[a]	Distance moved (mm)[b]	Time on disk (%)[c]
2	Water	24	14	58.3	15.9 ± 4.1[ab]	100.0 ± 0.0[a]
	Tween80	23	16	69.6	7.7 ± 2.3[b]	100.0 ± 0.0[a]
	Acetamiprid	14	7	50.0	5.0 ± 1.4[b]	100.0 ± 0.0[a]
	Pyrethrin	23	18	78.3	20.5 ± 3.9[a]	96.5 ± 2.1[b]
	Farnesol	19	12	63.2	27.6 ± 8.2[a]	100.0 ± 0.0[a]
24	Water	24	15	62.5	16.7 ± 6.2[b]	99.8 ± 0.2[a]
	Tween80	20	14	70.0	27.6 ± 8.4[b]	100.0 ± 0.0[a]
	Acetamiprid	15	9	60.0	4.7 ± 1.1[b]	100.0 ± 0.0[a]
	Pyrethrin	24	18	75.0	107.2 ± 25.9[a]	69.1 ± 9.4[b]
	Farnesol	18	15	83.3	7.7 ± 0.9[b]	100.0 ± 0.0[a]
48	Water	19	15	79.0[ab]	9.1 ± 2.6[c]	100.0 ± 0.0[a]
	Tween80	15	10	66.7[ab]	10.4 ± 3.0[bc]	100.0 ± 0.0[a]
	Acetamiprid	14	7	50.0[b]	9.4 ± 2.5[c]	100.0 ± 0.0[a]
	Pyrethrin	16	14	87.5[a]	36.9 ± 8.6[ab]	98.7 ± 1.0[a]
	Farnesol	15	13	86.7[a]	49.4 ± 16.6[a]	77.8 ± 10.0[b]

Control treatments were distilled water, Tween80 at 0.5% concentration (v/v), acetamiprid and pyrethrin, both at 1 L ha^{-1}. The natural active substance tested was farnesol mixed with Tween80 (1:2) nanoemulsion at 0.5% concentration (v/v). Different lowercase letters in the same column indicate significant differences among treatments by χ^2 2 × 2 contingency table or Kruskal–Wallis H test ($P \leq 0.05$)

[a] Percentage of aphids that moved during the 10-min recording out of total aphids evaluated, analyzed by χ^2 2 × 2 contingency table

[b]Total distance moved by aphids during the 10-min recording, analyzed by Kruskal–Wallis H test

[c]Percentage of time that aphids spent on the leaf disk during the 10-min recording, analyzed by Kruskal–Wallis H test

CABYV compared to water-mock (Table 3). Acetamiprid significantly reduced also the secondary spread of CABYV compared to water-mock ($\chi^2 = 5.0$, $P = 0.026$). Farnesol also reduced the secondary dispersion of CABYV by 40%, although no significant differences were obtained ($\chi^2 = 3.3$, $P = 0.068$).

Table 3 Primary and secondary spread of CMV and CABYV transmitted by *Aphis gossypii* evaluated 4 weeks after treatment application under greenhouse conditions

Virus	Spread	Treatment	Transmission rate (%)	Infected/total plants
CMV	Primary (24 h)	Water	11.6 a	17/147
		Acetamiprid	16.3 a	24/147
		Farnesol	14.3 a	21/147
	Secondary (7 days)	Water	36.3 a	49/135
		Acetamiprid	9.6 b	13/135
		Farnesol	38.2 a	50/131
CABYV	Primary (48 h)	Water	28.1 a	41/146
		Acetamiprid	8.8 b	13/147
		Farnesol	14.3 b	21/147
	Secondary (2 days)	Water	19.3 a	26/135
		Acetamiprid	9.7 b	13/134
		Farnesol	11.3 ab	15/133

Control treatments were distilled water and acetamiprid at 1 L ha^{-1}. The natural active substance tested was farnesol mixed with Tween80 (1:2) nanoemulsion at 0.5% concentration (v/v). Different lowercase letters in the same column indicate significant differences among treatments by χ^2 ($P \leq 0.05$)

Discussion

The rising demand for natural, highly selective and environmentally safe plant protection products has positioned extracts and EOs derived from plants as worthwhile alternatives to synthetic pesticides due to their particular properties (Isman 2006; Reyes-Jurado et al. 2020; López et al. 2021; Ferraz et al. 2022; EPA 2024; EU 2024). Weaknesses of biopesticides (low water solubility, high volatility, weak penetration, and persistence) can be overcome by the nanoformulation of the natural active ingredients (Klang and Valenta 2011; Shah et al. 2016; Pascual-Villalobos et al. 2017, 2019).

The phytotoxicity of melon plants was evaluated by visual inspection of symptoms and quantification of total chlorophyll content. Plants treated with farnesol exhibited brown circular spots and displayed malformations. Farnesol alters the biosynthesis of lipids and proteins and interferes with the homeostasis of intracellular calcium, which could explain the foliar phytotoxic symptoms observed (De Loof and Schoofs 2019). The reduced growth may be due to the interference of farnesol with the abscisic acid (ABA)

pathway. In this sense, *Arabidopsis thaliana* L. accumulating farnesol presents a delay in germination, growth, and floral development as a consequence of greater accumulation of ABA (Fitzpatrick et al. 2011). This accumulation could benefit melon crop in return, since plants accumulate ABA in response to the attack of pests and pathogens (Ton et al. 2006), which, added to its repellent effect, could explain the pesticide potential of farnesol.

Nanoemulsions of farnesol mixed with Tween80 or soy lecithin at 0.6–1% are reported to have high phytotoxicity on pepper and lettuce crops (e.g., drying of leaf tips, dry mottle spots in leaves) (Cantó et al. 2022b; Pascual-Villalobos et al. 2022). This occurs because nanoparticles expand in water due to their higher solubility so that the active ingredient spreads over the entire leaf surface (Werrie et al. 2020; López et al. 2021). Despite phytotoxicity is less pronounced when sunflower oil is used in the formulation (Pascual-Villalobos et al. 2022), in our experiments, the increase in phytotoxicity depended on farnesol dose and not on the surfactant. Aniseed nanoemulsion did not cause phytotoxicity to melon, as happened in lettuce crop (Cantó-Tejero et al. 2022c). A solid formulation could help improving this problem, as well as prolonging shelf life (López et al. 2021). Our starting parameters (small particle size and large negative Z-potential) give an indication of good emulsion stability after formulation (Pascual-Villalobos et al. 2019; López et al. 2021). Thus, we envision optimistic prospects of the steadiness of the nanoemulsions, although we cannot exclude the possibility of minor changes during storage. Further studies on formulation could provide some measure of temporal selectivity, as there is abundant opportunity to enhance persistence through formulation, all while allowing the slow release of these actives (Isman 2020).

The chlorophyll content of plants treated with the surfactant Tween80 was significantly higher than when treated with water-mock and with farnesol or aniseed nanoemulsions containing Tween80 at short term (4–11 days). The relatively large variability of chlorophyll content could be influenced by individual plant differences, inherent variation within plants, sample handling or instrument sensitivity, among others, highlight the need for further studies to minimize such variability. The disappearance of this effect over time (25 days) could indicate a potential recovery from the stress. There is no evidence that these outcomes produce a decrease in melon yield. Ultimately, testing the long-term impact of these compounds on the crop is highly advisable to rule out negative consequences of farnesol nanoemulsion on production in the field. Not being the initial objective of this study, the assessment of these biopesticides on the vegetative parameters, biochemistry, and yield of the crop is indeed a core research line included in a recently submitted funding proposal.

The behavioral response of wingless *A. gossypii* was evaluated on treated melon leaf disks. After 48 h of application, acetamiprid caused a reduction in the proportion of mobile aphids compared to pyrethrin and farnesol. The application of natural actives (pyrethrin and farnesol) caused aphids to walk more distance while they spent less time on the disk. In other words, aphids walked faster. The synthetic pesticide acetamiprid has a rapid knockdown effect that directly disrupts sodium channels in the nerve cells causing overstimulation (IRAC 2024). This leads to muscle spasms, hyperactivity, and uncoordinated movement. The physical exposure of the aphid body to pyrethrin might have triggered aphids to move rapid in order to escape toxicity. On the contrary, the mode of action of farnesol is not well-known. It negatively alters the settlement of aphids *R. padi* on barley, and *M. persicae* and *M. euphorbiae* on pepper (Pascual-Villalobos et al. 2017, 2019; Cantó-Tejero et al. 2022a), but not the feeding behavior of *A. gossypii* and *M. persicae* (Wróblewska-Kurdyk et al. 2020; Martín et al. 2024). Thus, the negative interaction of farnesol with *A. gossypii* settlement cannot be attributed to changes in insect feeding but it could be directly linked with olfactory or gustatory repellence, topical excitatory activity or post-feeding behavioral consequences, likely due to its chemical similarity to (E)-β-farnesene (EβF), an alarm pheromone that induces repulsion in aphids (Cantó-Tejero et al. 2022a; Martín et al. 2024).

Very few studies have investigated the effect of bioactive volatiles on plant viral transmission, and none is reported to evaluate nanoemulsions under semifield conditions. Among the scarce literature, a significant inhibition of tobacco mosaic virus (TMV, *Tobamovirus*) expression has been reported using tea tree oil nanoemulsions in vitro (Besati et al. 2024). Our results on the primary and secondary spread of melon viruses differed according to the mode of transmission (Hull 2014). On one hand, pesticide application before aphid release did not prevent the primary spread of CMV, regardless the nature of the active ingredient, either synthetic (acetamiprid) or natural (farnesol). CMV is efficiently acquired and inoculated in a non-circulative manner, during very brief (5–10 s) intracellular probes of insect vectors on the epidermal tissues without a latent period (Ng and Perry 2004). Short retention times go up to 12 h. In the primary spread experiment, viruliferous *A. gossypii* were able to land on a host plant and inoculate CMV during the first probes before pesticide action on the aphid, hence the same transmission rate as in control treatment. Only acetamiprid reduced the secondary spread of CMV. In our experiment, non-viruliferous aphids had to first land in a pesticide-treated CMV-infected source plant, acquire the virus, and then fly to another host to inoculate CMV. During this process, the rapid knockdown effect of acetamiprid targeted the

aphid nervous system and compromised its viability as a vector (IRAC 2024). The repellent effect of farnesol was insufficient to prevent CMV spread. In fact, EβF can even increase the transmission of a non-persistent, potato virus Y (PVY, *Potyvirus*), due to the induced activity and mobility in aphids (Lin et al. 2016).

On the other hand, farnesol and acetamiprid were able to reduce the spread of the persistently-transmitted CABYV. Transmission of persistently-transmitted viruses typically requires host colonization (Perring et al. 1999; Alonso-Prados et al. 2003). Increased vector mobility prevents the establishment of aphids on the host plants. Aphids need to feed from the phloem during a sustained period of time to acquire and transmit persistently-transmitted viruses (Hull 2014). They also need a latent period, a timespan between the virus acquisition by the vector and the ability to transmit the virus. Regardless their infectious status, either viruliferous or non-viruliferous, aphids were contaminated by pesticides during ingestion and died or had their activity compromised before subsequent CABYV transmission to healthy test plants. This is why the secondary spread of CABYV was reduced by 40% after farnesol treatment compared to water-mock control but remained on the edge of statistical significance. This outcome suggests that an extension of the IAP or an increase in the number of replicates we used would confirm the efficacy of farnesol on preventing the secondary spread of CABYV. Therefore, we could assume that, under real field conditions, where aphids are free to probe and feed for an unlimited time on their host plants, farnesol could be a viable alternative for CABYV control.

To sum up, the application of respectful alternatives to synthetic pesticides must be accompanied with an extensive insight of their individual biological features and impacts toward targeted pests and host plants. Pyrethrin and farnesol caused aphids to walk faster. Farnesol happened to be an option to manage CABYV spread, but not CMV. Therefore, the integrated management of *A. gossypii* transmitting non-circulative viruses should be reinforced with the introduction of natural enemies such as *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), whose activity and development are compatible with nanoformulations of farnesol and aniseed (Dáder et al. 2024).

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Author's contribution AF and AM provided funding. AF and AM conceived and designed research. JN-C and AT conducted experiments. MJP-V provided nanoformulations. BD and AM analyzed data. BD and AM wrote the manuscript.

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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. No datasets were generated or analyzed during the current study.

Declarations

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

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