Industrial Crops & Products 183 (2022) 114975

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Plant bioactive volatile products and their efficiency in aphid control

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A R T I C L E I N F O

Keywords:
Anise
Nanoemulsions
Essential oils
Cottonseed oil
Botanical insecticide
Myzus persicae

A B S T R A C T

The "farm-to-fork strategy" of the European Union aims to reduce dependency on pesticides and towards increased use of low-risk products, such as those based on botanical extracts. A product based on the mixture of the five natural compounds selected for their repellent or insecticidal properties, namely citral, *(E)*-anethole, farnesol, *cis*-jasmone and lemon essential oil, was evaluated against *Myzus persicae* Sulzer (Hemiptera: Aphididae). Three formulations were prepared using various surfactants at different ratios: the first one with Tween80, the second one with soy (*Glycine max* (l.) Merr.) lecithin and the third one with soy lecithin and sunflower oil (*Helianthus annuus* L.). The oil-in-water nanoemulsions at 1% were tested in a laboratory study with Petri dishes using a computer-controlled spraying apparatus (equivalent application of 200 l/ha). The mean efficacy was 45%, 71% and 63%, respectively, with a least statistically significant difference (LSD) at 5% comparing the first two formulations. A field experiment (in two pepper, *Capsicum anuum* L., greenhouses) at Torreblanca Exp. Stat. in Murcia (Spain) followed in April 2020 in which a reduction in aphid populations only occurred by spraying the product formulated with soy lecithin. Phytotoxicity was also observed but was lower in the formulation that contained sunflower oil. Nanoemulsions were characterised using a Zetasizer, and a polydispersion of 2–3 populations of particles, ranging from 15 to 341 nm in size, was found with the Tween80 formulation and bigger sizes (250–438 nm) with the soy lecithin and sunflower oil formulation, which were more stable (Z potential = −28.15 mV). The application of ultrasounds reduces the Z-average to 100 nm in the mixture product with stability for at least 14 days. Another field experiment was repeated in February and April 2021. The plants were sprayed sequentially with the following: a) mixture product at 0.5% formulated with soy lecithin and sunflower oil, b) 3% cottonseed (*Gopsyppium hirsutum* L.) oil in 3% soap water (Feb.) or 1.5% cottonseed oil in 1.5% soap water (Apr.) and c) *cis*-jasmone at 0.25% formulated with Tween80. The treatment with the mixture of bioactive volatiles (a) was not more effective than that of the fixed seed oil (b). In February, the instantaneous population growth rate (ri) of aphid populations showed a significant reduction when the plants were sprayed with cottonseed oil. Further research is recommended for the formulation and application methods of the products being developed.

1. Introduction

Industrial non-food crops are the source of valuable products that can be used as raw materials in botanical insecticides. Essential oils (EOs) are produced in spices such as anise (*Anethum graveolens* L.) or in waste streams, for instance, in the citrus industry. The selection of active compounds is relevant in research to develop novel applications, but the industry generally uses the available and cheap raw materials. In recent years, aphids have emerged as critical pests for pepper grown under greenhouse conditions in Southeast Spain, causing direct and indirect (due to virus transmission) damage to the crop (personal communication). Chemical agriculture is withdrawing many active substances and is experiencing increasingly fewer effective compounds and resistance is developing in pests (personal communication). Mineral oils are a common active ingredient used in organic and zero-residue agriculture nowadays, but there is a need to find alternatives, and plant vegetable oils and/or plant extracts are good candidates for this purpose.

Oil in water (O/W) emulsions are receiving attention because of the need to reduce the volatile organic solvents used in emulsifiable concentrates. Nanoemulsions are those in which the particles are in the

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https://doi.org/10.1016/j.indcrop.2022.114975
Received 16 September 2021; Received in revised form 5 April 2022; Accepted 7 April 2022
Available online 27 April 2022
0926-6690/

nanometric scale. The small size of oil droplets provides some interesting properties, such as activity at lower doses or penetration enhancement (Pascual-Villalobos et al., 2017b). Mondal et al. (2017) have published that azomethine-based nanofungicides are twice as effective as conventionally sized azomethines. The surfactants and stabilisers of the formulations play a relevant role in the characteristics and stability of the emulsions. The surfactants adsorb to oil droplet surfaces, reducing the interfacial tension that causes disruption and aggregation, therefore stabilising the emulsions. In some cases, the use of surfactant mixtures (or cosurfactants) improves the preparations by reducing the interfacial tension and increasing the fluidity of the interface. Limpongsa et al. (2019) show the case of grapefruit (*Citrus paradisi* Macfad.) oil and caffeine in cosmetics. There is a tendency to replace synthetic surfactants with natural ones (Bai and McClements, 2016; Schreiner et al., 2020). Among the natural surfactants, we can mention lecithins and saponins. Plant lecithins are complex mixtures of phospholipids and retain oil soluble substances in the lipid bilayer membrane. They are registered as basic substances in EU regulations and can therefore be used in the formulation of plant protection products. Saponins are isolated from the bark of the tree *Quillaja saponaria* Molina (Quillajaceae) or from tea (*Camelia sinensis* (L.) Kuntze, Theaceae) seeds, and rhamnolipids are biosurfactants derived from microorganisms (Bai and McClements, 2016). Natural surfactants from plants can replace synthetic surfactants such as Tween80 and can form and stabilise nanoemulsions. Zhu et al. (2018) reported that with orange (*Citrus sinensis* Osbeck) oil, particle diameters are less than 150 nm. Schreiner et al. (2020) have published that *Quillaja* saponins are more effective alone than in combination with glycerol to form nanoemulsions of sweet almond (*Prunus dulcis* Mill. D.A. Webb.) oil.

In previous works (Pascual-Villalobos et al., 2017a; Cantó-Tejero et al., 2021a), we selected some bioactive compounds against *Myzus persicae* Sulzer (Hemiptera: Aphididae) for their repellent or insecticidal properties, namely the following: citral (the blend of two isomers, neral and geranial), *(E)*-anethole (the main compound of aniseed EO), farnesol, *cis*-jasmone and lemon EO (which contains mainly limonene). According Cantó-Tejero et al. (2021a), the natural compounds farnesol, *cis*-jasmone and *(E)*-anethole are the most *M. persicae*-repellent compounds. The foliar applications of farnesol plus *(E)*-anethole at 0.2% each reduced the aphid population growth rate, ri calculated as per the conception of Ribeiro et al. (2014), (ri = −0.78 at 24 h), and blending both products increased the insecticidal activity although no synergistic effects were obtained. *Cis*-jasmone also had an effect on aphid mortality.

The objective of this work was to characterise nanoemulsions of a product developed through a mixture of active plant compounds and formulated with synthetic or natural surfactants and to test its insecticidal effect in field experiments using the aphid (*M. persicae*) / pepper crop as a real reference system of agricultural production in Southeast Spain.

2. Materials and methods

2.1. Nanoemulsion preparation and characterisation

Five plant-based volatile products – citral, *(E)*-anethole, farnesol, cis-jasmone and lemon (*Citrus limon* L.) EO – were used in the experiments. The first four were purchased from Sigma Aldrich (St. Louis, MO, USA), and the fifth was obtained from Citromil S.L. (Santomera, Murcia, Spain). The mixture product combined the five of them in equal amounts. Three formulations were prepared using a high-speed rotor IKA Labor Pilot 2000/4 (IKA-Werke GmbH and Co. Staufen, Germany) (10 min, 7940 rpm, 15ºC): the first one with Tween80 1:2, the second one with soy lecithin 1:1 and the third one with soy lecithin and sunflower oil 1:1:1. The emulsions were prepared at 5% and then diluted. The nanoemulsions were characterised (particle size distribution and Z potential) using a Zetasizer Nano ZS (Malvern Panalytical Ltd.) and a Zetasizer Advance Ultrared (MADLS). In order to test the stability of the samples in storage, a Digital Branson Ultrasonicator 4500 with a tapered disruptor horn of ½” 400 W was utilised with 30% amplitude for 5 min with 15 s pulses.

2.2. Insects

A culture of *Myzus persicae* Sulzer (Hemiptera: Aphididae) (a clone with pink colour) was reared in our laboratory on pepper plants (*Capsicum annuum* L., cv. Herminio) in a growth chamber under a 16:8 (L:D) photoperiod at 25 ± 1ºC and 65% relative humidity. Apterous adult females (unsyncronized) were used in the laboratory assay as well as to inoculate plants in the field experiments. Plants were left to evolve to check that before the treatments the number of aphids per plant was not statistically significant different.

2.3. Laboratory tests of aphicidal activity

The oil-in-water nanoemulsions of the mixture product at 1% (which contained 0.2% of each of the five pure compounds) were tested in a laboratory study with Petri dishes. The formulations evaluated were 1:2, 1:1 and 1:1:1; see Subsection 2.1 for details. The experimental unit comprised one Petri dish (5 cm diam.) with a pepper leaf on agar and approximately 20 settled aphids. There were thirty replications per treatment. The dishes were sprayed with a computer-controlled spraying apparatus (CCSA, Burkard Ltd.) at 0.345 bar and 6 twists (equivalent application of 200 l/ha). The number of alive and dead aphids was recorded after 1 and 4 days. Data were transformed by arcsin square root prior to analysis. The Henderson-Tilton mean efficacy (of each treatment against the corresponding control) was computed together with the LSD to compare the treatments statistically.

2.4. Field experiments

Experiments were conducted for two years at Torreblanca Exp. Stat. in Murcia (Spain), in 2020 and 2021. The methodology used is summarised in Table 1. The effect of spray applications on aphid populations was studied by statistical methods. In 2020, data of the number of alive and dead insects 4 days after treatment were analysed by ANOVA. In addition 95% LSD or box and whisker plots were used to compare

Table 1
Field experiments (pepper crop cv. Herminio) with bioactive volatile product applications. Methodology.

Year 2020	Year 2021
2 greenhouses Planting date: 27 January 2020 Month of experiment: April	2 greenhouses Planting date: 21 October 2020 Months of experiment: February and April
Aphid population before spraying: 28–200 aphids/plant	Aphid population before spraying: 20–50 aphids/plant
Randomised block design with 2 blocks	Randomised block design with 2 blocks
Experimental unit: 5 adjacent plants in the same row	Experimental unit: 2 plants in adjacent rows
Treatments[a] and controls (formulations 1:2, 1:1 and 1:1:1) of mixture product at 1%	Sequential treatments[a] and controls: a) mixture product (formulation 1:1:1) at 0.5%, b) 3% cottonseed oil in 3% soap water (Feb.) or 1.5% cottonseed oil in 1.5% soap water (Apr.) and c) cis-jasmone (formulation 1:2) at 0.25%
Data and analysis: Number of alive insects before the treatment and number of alive and dead insects after 1 and 4 days. ANOVA, LSD and Box and Whisker plots.	Data and analysis: Number of alive insects before the treatment and number of alive and dead insects after 1 and 4 days. ANOVA and Wilcoxon test.

[a] Mixture product: O/W nanoemulsion of a mix of citral, (*E*)-anethole, farnesol, *cis*-jasmone and lemon essential oil in equal amounts. Formulation 1:2 with Tween80, 1:1 with soy lecithin and 1:1:1 with soy lecithin and sunflower oil. Controls contained just the corresponding surfactants and water.

formulations with their controls. In 2021, data of the number of alive and dead insects were analysed by ANOVA and also by Wilcoxon test to compare each treatment with its control. The instantaneous rate of growth increase (ri) of aphid populations (calculated as per the conception of Ribeiro et al., 2014) was analysed as well.

3. Results

3.1. Characterisation of nanoemulsions

In Table 2, a summary of the results is shown. A polydispersion was found of 2–3 populations of particles ranging from 10 to 341 nm in size with the 1:2 formulation as well as bigger sizes (250–434 nm) with the 1:1:1 formulation, which had a more stable (Z potential = −28.78 mV) product. The polydispersity index (PdI) was within the range of 0.401–0.753 for the 1:1:1 formulation and 0.298–0.802 for the 1:2 formulation. Such values indicate polydisperse samples except for the control with just Tween80 (formulation 1:2) and the dilutions 2%, 1% and 0.25% in which PdI < 0.3. The particle concentration (no./ml) for the nanoemulsions at 1% ranged from 8.1 × 10^{10} to 7.0 × 10^{15} in the samples that were measured.

In Fig. 1A, the particle size distribution for the nanoemulsion of the mixture product (formulation 1:1:1) at 1% indicates main peaks of particles 490 nm and 143 nm in size, while the control has one main peak of 295 nm particles. In both cases, however, less frequent populations of particles, over 3500 nm in size, were present.

In addition, it was observed that cis-jasmone presented a good emulsion with Tween80 (1:2), with 15 nm particles and a PdI = 0.289 (compared to 353 nm and PdI = 0.486 of the formulation 1:1:1), see Fig. 1B.

The application of ultrasounds (Fig. 2) reduces the Z-average from 252 nm to 100 nm in the mixture product (formulation 1:1:1) with stability for at least 14 days. Therefore, in order to fabricate nanoemulsions with smaller particle sizes, the application of ultrasounds offers an advantage.

3.2. Aphicidal activity in laboratory tests

Fig. 3 shows the efficacy of formulations 1:2 (Tween80), 1:1 (soy lecithin) and 1:1:1 (soy lecithin and sunflower oil); the Henderson-Tilton efficacy for each was 45%, 71% and 63%, respectively, with an LSD statistically significant at 5% for the first compared to the last two formulations (Fig. 4). In the Petri dishes bioassay, the mixture product had a better insecticidal effect when soy lecithin was used in the formulation.

3.3. Field experiments

3.3.1. Year 2020

The field experiment (in two pepper greenhouses) in April 2020 resulted in a reduction in aphid populations by spraying the mixture product at 1% formulated with soy lecithin (1:1); therefore, this was consistent with the results of the efficacy test in the laboratory (see Subsection 3.2). In Fig. 5, we see the statistically significant difference between the number of alive insects in the treatment and control groups four days after spraying (formulation 1:1). The Kruskal–Wallis test (K =

Table 2
Characterisation of O/W nanoemulsions of the mixture product[a].

Product	Concentration (%)	Z-average (nm)	PdI	Peaks[b] (%)	Size (nm)	Pot Z (mV)	Particles/ml
Formulation 1:1:1 (with soy lecithin and sunflower oil)							
Mixture[c]	5	434 ± 10.9	0.725	85	559		
				15	4671		
	1	254 ± 3.132	0.429	55	490	-28.78	7.0 × 10^{15}
				39	143		
				6	4733		
	0.25	216 ± 2.974	0.401	84	259		
				16	2474		
Control[d]	5	324 ± 16.918	0.512	80	424		
				13	4035		
				7	61		
	2	394 ± 19.568	0.753	42	591		
				37	180		
				21	4541		
	1	250 ± 8.876	0.533	82	295	-28.15	8.1 × 10^{10}
				18	3550		
	0.25	282 ± 1.531	0.692	76	324		
				24	4118		
Formulation 1:2 (with Tween80)							
Mixture[c]	5	303 ± 12.319	0.729	80	336		
				7	74		
	1	70 ± 7.069	0.802	75	179	-17,17	5.8 × 10^{12}
				20	16		
	0.25	201 ± 61.768	0.298	63	161		
				37	15		
Control[d]	5	341 ± 21.764	0.667	85	405		
				8	5169		
	2	10 ± 0.194	0.294	87	9		
				10	650		
	1	10 ± 0.044	0.268	88	10	-7.29	3.7 × 10^{15}
				12	1452		
	0.25	121 ± 92.442	0.298	83	9		
				15	285		

[a] Particle size and PdI were measured with a Zetasizer Nano ZS. Z potential and particles/ml were measured with a Zetasizer Advance Ultrared (MADLS).
[b] Only peaks with > 5% in intensity were included.
[c] The mixture contained five bioactive products (*(E)*-anethole, *cis*-jasmone, citral, farnesol and lemon essential oil) in equal amounts. The emulsions were prepared at 5% and then diluted.
[d] Control was prepared the same, except the bioactive products.

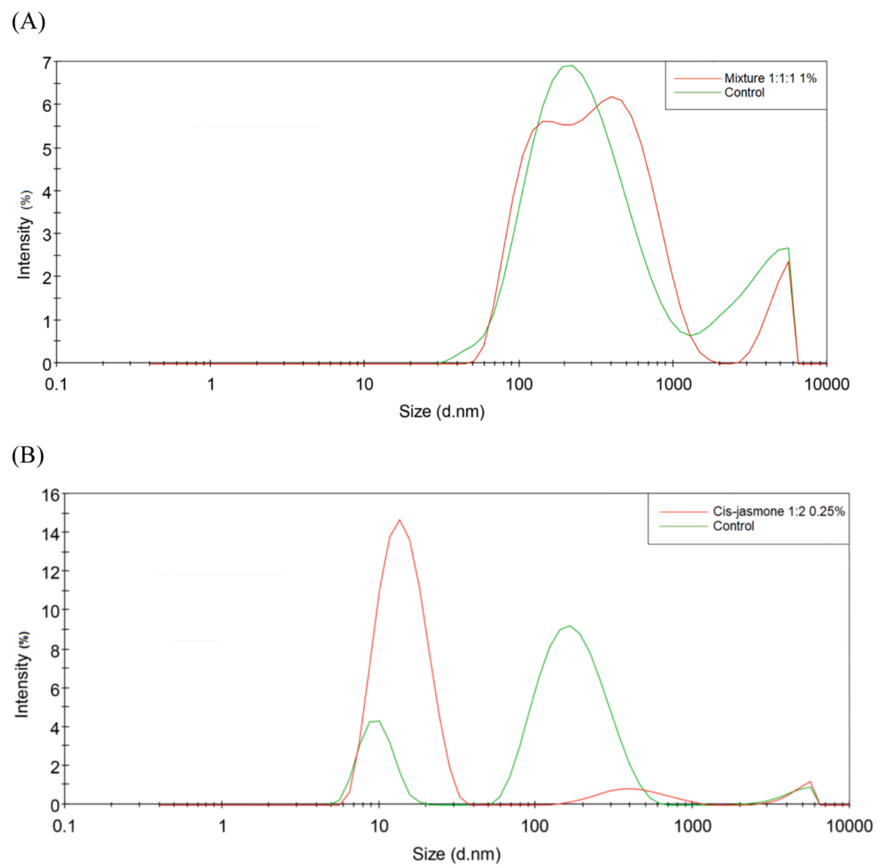

Fig. 1. Particle size distribution by intensity. (A) Mixture product at 1% and control nanoemulsions with formulation 1:1:1. (B) Cis-jasmone at 0.25% and control nanoemulsions with formulation 1:2.

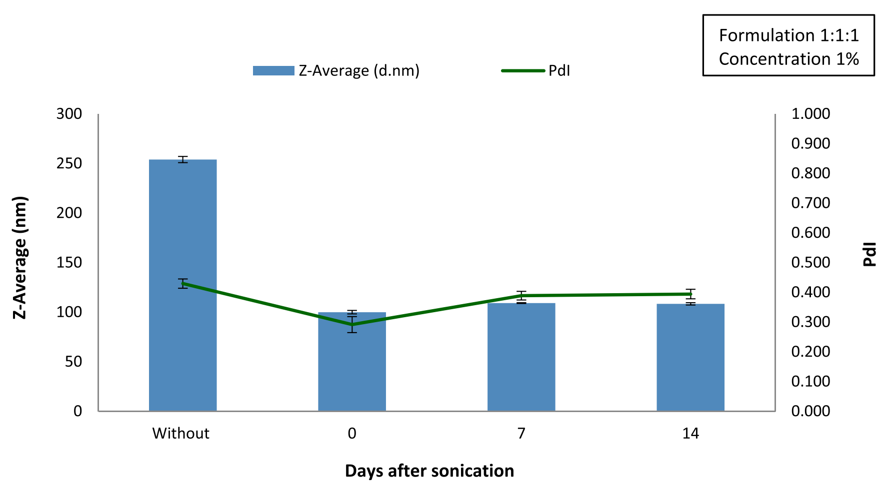

Fig. 2. Z-average and polydispersity index (PdI) by DLS of nanoemulsions of the mixture product (formulation 1:1:1) at 1% with and without application of ultrasounds and storage for seven or fourteen days.

5.451, df =1, $P = 0.0195$) was significant. No differences were obtained for the other two formulations (Fig. 5). Mortality was also increased by spraying the product (in all three formulations); however, a larger number of dead aphids were recorded with the Tween80 formulation (1:2). See the plate in Fig. 6 of a pepper leaf with the dead aphids. The Box and Whisker plot in Fig. 7 shows the statistically significant difference in the number of dead aphids between treatment and control (formulation 1:2) four days after spraying (F = 18.62 **). No differences were obtained for the other two formulations (Fig. 7). Phytotoxicity was also observed in the crop: slight drying of leaf tips and flower petals in some cases or dry mottled spots in the leaves. However, no damage was observed in the pepper fruits. Such symptoms were evident with both the 1:2 and the 1:1 formulations but not with the 1:1:1 formulation. If the application dose was reduced from 1% to 0.5% or even 0.25%, there was less insecticidal effect and phytotoxicity. Therefore, the 1:1:1 formulation (soy lecithin and sunflower oil) was selected for the field experiments in the following year (2021), as it had a balanced performance with a reduction in aphid populations and less phytotoxicity for the crop.

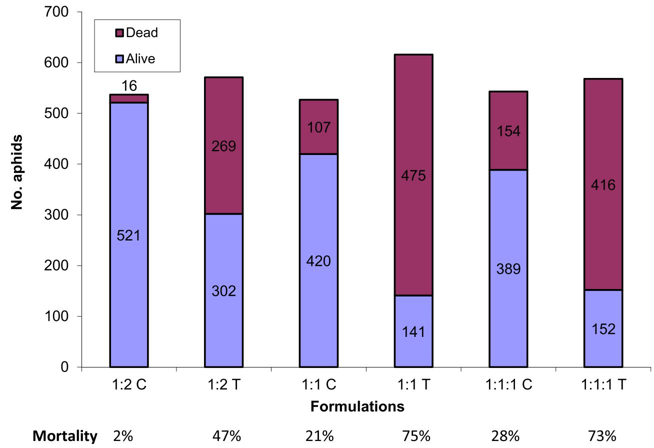

Fig. 3. Number of alive and dead adults and percentage of mortality four days after spraying O/W nanoemulsions of the mixture product at 1% with 3 formulations to Petri dishes with *Myzus persicae* (n = 30 and approximately 20 aphids/dish).

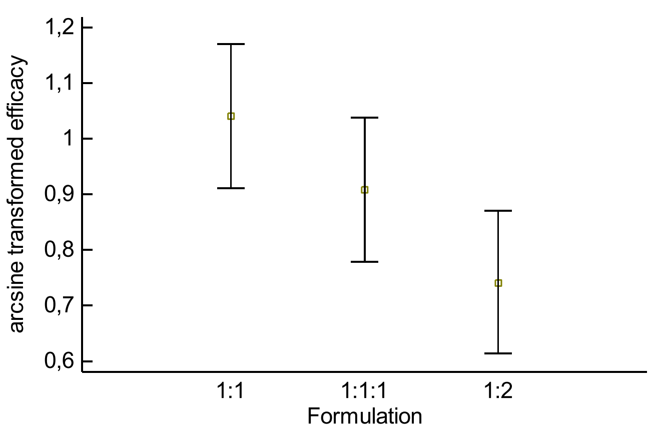

Fig. 4. Means and 95% LSD Intervals of the data (arcsine square root transformation), four days after treatment, from the Petri dishes with *Myzus persicae* settled on pepper leaf and sprayed with the mixture product at 1%.

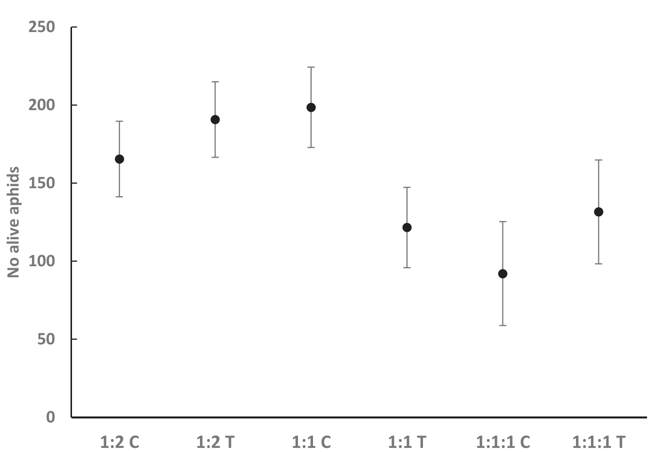

Fig. 5. Field experiment 2020: means and 95% LSD intervals of number of alive aphids (*Myzus persicae*) 4 days after treatment with the mixture product at 1% with 3 formulations.

3.3.2. Year 2021

For the field experiment in 2021, in two pepper greenhouses, we infested plants with aphids, but the initial populations were kept lower (20–50 aphids/plant) than the previous year (28–200 aphids/plant), otherwise the pest populations would increase exponentially, making it difficult to draw any conclusion regarding the effects of the treatments. In Fig. 8, an overview of the results is graphed for February and April. The same plants were sprayed sequentially (every 4 days) with the following: a) mixture product formulation 1:1:1 at 0.5% applied to the air (not directly to the plants to avoid phytotoxicity), b) 3% cottonseed oil in 3% soap water (Feb.) or 1.5% cottonseed oil in 1.5% soap water (Apr.) (directly to the plants) and c) *cis*-jasmone formulation 1:2 at 0.25% (directly to the plants). The corresponding controls were treated in the same manner except for the application of bioactives. In February, a reduction in alive aphids throughout the observation period was obtained for the treatments in comparison to the controls, whereas there was no insecticidal effect after the first treatment in April but was later noted after the second and third treatments. Overall, the first treatment, with the mixture of bioactive volatiles at 0.5% sprayed to the air (a) to reduce pests while avoiding phytotoxicity, was not statistically different from its corresponding control, and neither was it different from that of the vegetable fixed oil sprayed on the plants (b).

In February, the instantaneous rate of growth increase (ri) of aphid populations showed a significant reduction when the plants were sprayed with the cottonseed oil: the Wilcoxon test presented $W = 64$ ($p = 0.0009^{***}$) and ANOVA gave $F = 48.8$ (d.f. = 1, 13; $p < 0.000009$); in April, there was no statistically significant difference but it was close to 5% ($W = 49$, $p = 0.083$ and $F = 4.12$, d.f. = 1, 13; $p = 0.0633$). In addition, *cis*-jasmone spraying (c) in February (albeit with data variability) produced aphid mortality as well ($W = 7$, $p = 0.0322^*$ and $F = 5.28$, d.f. = 1, 13; $p = 0.0422$).

Table 3 presents data on average aphid mortality. In February, an aphicidal effect was produced in all treatments with mortality values over 80% after spraying with cottonseed oil at 3% or *cis*-jasmone at 0.25%. In April, such effects were much lower but with a statistically significant difference for cottonseed oil at 1.5%.

Fig. 6. Aphid mortality spraying O/W nanoemulsions of the mixture product at 1%.

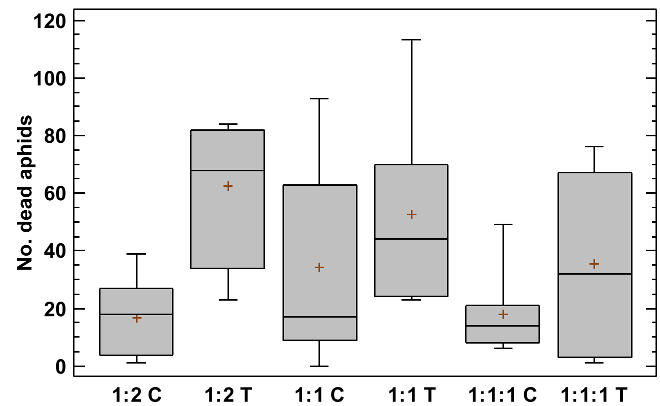

Fig. 7. Field experiment 2020: Box and Whisker plot of dead aphids (*Myzus persicae*) 4 days after treatment with the mixture product at 1% with 3 formulations.

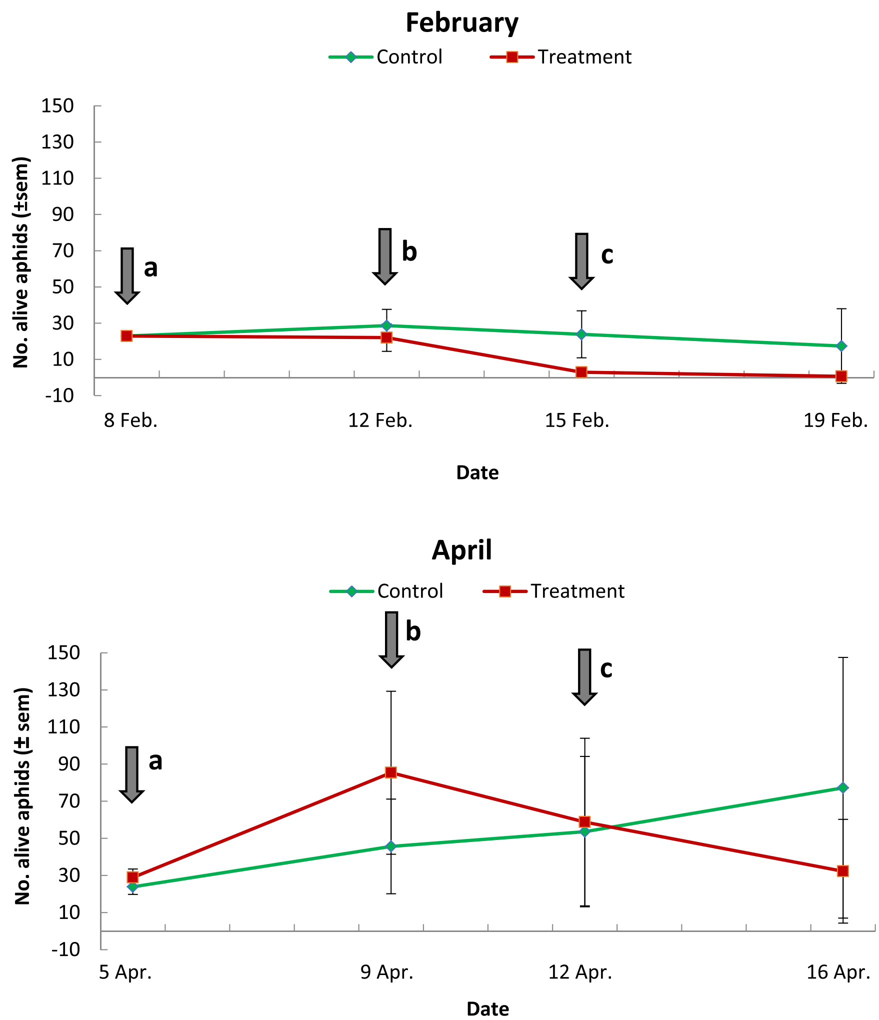

Fig. 8. Field experiment 2021 (pepper crop cv. Herminio) with sequential spraying of treatments: a) mixture product (formulation 1:1:1) at 0.5%, b) 3% cottonseed oil in 3% soap water (Feb.) or 1.5% cottonseed oil in 1.5% soap water (Apr.) and c) *cis*-jasmone (formulation 1:2) at 0.25%.

Table 3
Aphid mortality (%) (± SEM) after 4 days of application (Field experiment 2021).

	February		April	
	Treatment	Control	Treatment	Control
Mixture product	30.3 ± 8.49	24.1 ± 6.54	0 ± 0	1 ± 0.63
Cotton seed oil	82.0 ± 5.40***	18.3 ± 2.69	20 ± 5.32**	2 ± 1.97
Cis-jasmone	83.3 ± 16.67*	9.7 ± 2.61	14 ± 12.29	1 ± 1.03

Wilcoxon test to compare treatment and control. Values for statistically significant treatments: W= 0 (P = 0.000931) or W= 4 (P = 0.002571) for cotton seed oil in Feb. or Apr. and W= 7 (P = 0.02862) for cis-jasmone in Feb.

4. Discussion

The particle size of nanoemulsions depends on the respective bioactive compounds, surfactants and stabilisers used and the method of fabrication. Riquelme and Arancibia (2019) reported that saponins improve PdI and Z potential but are less effective at reducing particle size. In our experiment, if fixed oils were used – e.g. sunflower oil in the 1:1:1 formulation or glycerol in the 1:2:1 formulation previously reported (Pascual-Villalobos et al., 2017a) – the overall particle size increased, but the stability of the product improved as well. On the other hand, the use of ultrasound resulted in smaller sizes and stability over time. Increasing the amount of surfactant is a way to reduce the particle size of a nanoemulsion (Jaworkska et al., 2013); however, an excessive amount may be detrimental to the delivery of entrapped bioactives, as Chuacharoen et al. (2019) indicated for curcumin in milk.

Mixing several compounds in one product may offer some advantages such as in the case of combining insecticidal, repellent or other effects in a botanical agent, but it can potentially make obtaining a monodisperse nanoemulsion more complicated. The hydrophilic lipophilic balance (HLB) of the oil phase has to be obtained experimentally, and the choice of surfactants should match such a value for a stable emulsion. Parra Barona (2018) tested different mixtures of Tween80 and Span80 with Sacha inchi (*Plukenetia volubilis* L.) oil. The encapsulation of a bioactive is important in formulations; for instance, Ge and Ge (2015) pointed out that for *Melaleuca alternifolia* Maiden & Betche ex Cheel (tea tree) oil, preservation from light, oxygen and temperature is required and, in addition to that, a slow release is produced compared to unformulated tea tree oil. Another advantage of mixing compounds is reducing the Ostwald ripening (the solubilisation of small oil droplets in water due to the high Laplace pressure) which increases the size of emulsion particles over time, because some of them may act as ripening inhibitors.

Weekly sprays of citral or anise essential oil at 0.2% prevented natural infestations of aphids in a pepper crop, and curative applications of the two products together reduced aphid populations (Pascual-Villalobos et al., 2019). Such products, although less effective than pyrethrum (*Tanacetum cinerariifolium* Trevir. Sch. Bip.), proved to be safer for natural enemies. Other authors (Erlina et al., 2020; Lina et al., 2020) have also presented insights on botanical insecticides: the nanoemulsion of *Piper aduncum* L. (Piperaceae) fruit (which contains the phenylpropanoid dilapiol) at 0.5–5% has residual toxicity in the cabbage larvae *Crocidolomia pavonana* F. (Lepidoptera: Crambidae), reducing the growth of the larvae.

The literature reports that farnesol or (*E*)-anethole nanoemulsions are more active (Repellency Index of 47.1–60.8 or 25.8–59.6, respectively) if formulated with soy lecithin (Pascual-Villalobos et al., 2019). In the field experiments presented herein, the reduction in aphid populations was clear when soy lecithin was used in the formulation of the bioactive mixture product spray at 1%. The mortality of aphids tended to be greater for formulations with Tween80 or soy lecithin, but crop phytotoxicity was more pronounced in those cases as well. However, when sunflower oil was used in the formulation, there were much fewer phytotoxic symptoms. To slow down the spread of aphids in the crop by applying the bioactive volatile product, we had to avoid large populations (over 200 aphids/plant) as a starting point because botanical products do not produce 100% mortality and the insects remaining alive propagate fast in numbers. In addition, earlier applications in the season (winter) have more efficacy than those later in spring when we would have the optimal environmental conditions for fast aphid reproduction.

In other experiments, we did not obtain phytotoxic effects in pepper if the bioactive volatiles were sprayed at 0.2%. Recently, Cantó-Tejero et al. (2021b) observed that application of *(E)*-anethole against *Nasonovia ribisnigri* Mosley (Hemiptera: Aphididae) in lettuce at doses of 0.2–0.4% presented efficacies over 43% without phytotoxicity and allowed the presence of biological control agents (ladybirds and syrphids) that were not seen in the plots treated with pyrethrum.

Our results indicate that sprays of *cis*-jasmone at 0.25% have aphicidal activity. In previous works (Pascual-Villalobos et al., 2017a), we obtained repellency (R.I. = 58.6) of cis-jasmone to *Rhopalosiphum padi* L. (Hemiptera: Aphididae) at 0.02 $\mu l/cm^2$.

The tested product of bioactive volatiles has potential, but there is room for improvement concerning the formulations. So far, the application of fixed oils (e.g. cottonseed oil tested in the field experiment in 2021) as an insecticidal spray was better, but new alternatives are needed and will be pursued by our group in future research.

5. Conclusions

The plant bioactive volatile products tested present promising efficacy against aphids. Although formulations with soy lecithin and Tween80 were more active than the formulation with sunflower oil and soy lecithin, the latter one was safer to spray on the pepper crop to avoid phytotoxicity.

More research is needed on the formulation and application method to produce better results in aphid control and to eliminate the phytotoxic side effect being observed. The selection of the appropriate surfactants depends on the active ingredient properties and should provide small particle sizes, a low PdI, a large Z potential value and maintenance of its characteristics after dilution.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work has been funded by the National Institute for Agricultural and Food Research and Technology (INIA) with the project RTA2017-00022. Manuel Cantó and Félix Martín were awarded the predoctoral scholarships INIA CPD2016-0092 and INIA PRE2018-084296, respectively.

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