



Cite this: *Environ. Sci.: Nano*, 2025, 12, 5257

Is natural always safe? Effective botanical nano-aphicide can be harmful to pollinators

Antonino Modafferi,^a Ilaria Latella,^{iD}^a Marianna Longordo,^a Maria Pineda,^{iD}^a Raffaele Cavaliere,^{bc} Pierluigi Forlano,^b Vincenzo Palmeri,^a Giulia Giunti^{iD}^{*b} and Orlando Campolo^a

Among the innovative and eco-friendly solutions to conventional pesticides, nano delivery systems (*i.e.*, nanostructures and nano-emulsions) seem to be ideal candidates for botanical formulations to be used as insecticides. In this context, the proposed study aimed to formulate an *Allium sativum* EO-based nano-emulsion and to evaluate its toxicological activity against *Aphis gossypii*. Furthermore, the adverse effects of the nano-formulation on honeybees and plants were also investigated. The chemical composition of the garlic EO highlighted that the EO was composed only of sulfur compounds (95.35% of the total area). The nano-formulation (15% EO; 5% Tween® 80; 80% water w/w/v) was obtained using high-pressure microfluidization (HPM) techniques and physically characterized by dynamic light scattering (DLS). The results highlighted optimal physical properties with particle sizes ranging in the nanoscale (179 ± 1.4 nm), good polydispersity indices (PDIs), with values inferior to 0.25, and negative surface charge after 1 month of storage. The toxicological bioassays against the target pest showed high insecticidal activity with low estimated lethal doses in both residual contact toxicity (LD₃₀ of 0.810, LD₅₀ of 1.079, and LD₉₀ of 2.171% of EO) and topical application (LD₃₀ of 0.133, LD₅₀ of 0.212, and LD₉₀ of 0.667% of EO) after 72 h exposure. While negligible phytotoxic effects on sweet pepper plants were detected, the developed EO nano-emulsion revealed high toxicity towards honeybees through ingestion application. Overall, this study proved the high efficacy of the developed nano-biopesticide against the target pest; however, further studies are needed to fully understand the impact of these new nano-insecticides on non-target organisms in agroecosystems.

Received 20th May 2025,
Accepted 3rd October 2025

DOI: 10.1039/d5en00498e

rsc.li/es-nano

Environmental significance

Recently, nanotechnologies have found wide application for biopesticide formulations, since these allow several shortcomings of botanical extracts to be overcome while improving their effectiveness against target pests. The safety of nano-bioinsecticides toward both the environment and non-target organisms is not questioned and generally assumed. Nevertheless, nano-systems could deeply change the biological activity of botanical active substances, affecting either target or non-target species, including pollinators. Here, a nano-emulsion containing garlic essential oil showed good insecticidal activity against a target aphid species, although it also caused severe mortality toward honeybees by ingestion. In this scenario, a deeper understanding of the ecotoxicological impact of nano-biopesticides is needed to correctly design integrated pest management programs avoiding negative effects on pollinator populations.

1. Introduction

Aphis gossypii (Hemiptera: Aphididae), commonly known as the cotton aphid, is a polyphagous species that causes direct damage such as plant weakening, leaf yellowing, wilting, and the secretion of honeydew, which promotes sooty mold and hinders photosynthesis, and indirect damage (*i.e.*, pathogen

transmission) to several plant families (*i.e.* Rutaceae, Malvaceae, Cucurbitaceae, *etc.*).^{1–3} To date, the main control strategies are based on the use of conventional synthetic pesticides, which resulted in various consequences on the environment and human health, and also negative effects on several non-target organisms, including pollinators, natural antagonists, aquatic organisms, and invertebrates.^{4,5}

Among the green solutions, botanicals such as aqueous extract, oil, and essential oils (EOs) stand out as ideal candidates to replace conventional pesticides due to their proven insecticidal activities.^{6–8} In this regard, several authors have well documented the effectiveness of EOs in managing aphids.^{9–13} As reported by Koorki *et al.* (2018),¹⁴ *Eucalyptus camaldulensis* Dehn., *Rosmarinus officinalis* L., and

^a Department of AGRARIA, University of Reggio Calabria, Loc. Feo di Vito s.n.c., Reggio Calabria 89122, Italy

^b Department of Pharmacy, University of Salerno, Via Giovanni Paolo II 132, Fisciano 84084, SA, Italy. E-mail: ggiunti@unisa.it

^c Department of Agricultural, Forest, Food and Environmental Sciences, University of Basilicata, Via dell'Ateneo Lucano 10, Potenza 85100, Italy



Ferula assa-foetida L. EOs showed repellency and fumigant toxicity against 2-day-old nymphs of *A. gossypii* 24 hours after exposure with estimated lethal concentrations (LC)₅₀ of 21.02, 19.28, and 4.64 $\mu\text{L L}^{-1}$ air, respectively. Furthermore, several EOs could significantly alter nymphal development, as well as adult longevity, fecundity, and the amount of honeydew produced by this pest.¹⁵ Moreover, Albouchi *et al.* (2018)¹⁶ investigated the biological activity of an EO extracted from *Melaleuca styphelioides* Sm. against three citrus aphids, including *A. gossypii*, *Aphis spiraeicola* Patch, and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), highlighting that this EO exhibited effective fumigant and contact toxicity against all test species, although *A. gossypii* was the least susceptible species.

Among botanicals, *Allium sativum* L., commonly known as garlic, has been widely used in pest control due to its high toxicity. In this context, the topical application of garlic oil against adult *A. gossypii* exhibited good insecticidal activity with an estimated LC₅₀ and LC₉₀ of 3302 and 10553 ppm, respectively, 48 hours after exposure.¹⁷ Using the same application method, Aiad *et al.* (2019)¹⁸ highlighted high insecticidal activity against *A. gossypii* with an estimated LC₅₀ of 0.59 mg L⁻¹ and LC₉₀ of 1.24 mg L⁻¹. Furthermore, the application of 10 μL of garlic oil led to high mortality (100%) against *M. persicae*.¹⁹ In addition, the application of garlic EO (3% concentration) could reduce the aphid population by 100% after 1 week of treatment.²⁰

Despite the high efficiency of these botanicals in pest management, some drawbacks (*i.e.*, flammability, poor solubility in water, rapid degradation of the active ingredient, and volatility) limit their use under real-operating context.^{21,22} To overcome these drawbacks, in the last decade, the use of nanotechnologies to develop nano-delivery systems (*i.e.*, nanostructures and nano-emulsions) has increased the interest of researchers.^{23–25} The high biological activity of innovative nano-insecticides has been well documented against several pests.^{26–30} For instance, Tortorici *et al.* (2022)³¹ developed three different nanostructured lipid carriers (NLCs) loaded with different EOs (*i.e.*, lavender, rosemary, and peppermint) and tested the biological activity against *A. gossypii*, *Spodoptera littoralis* (Bois) (Lepidoptera: Noctuidae), and *Phthorimaea (Tuta) absoluta* (Meyrick) (Lepidoptera: Gelechiidae). The results highlighted good insecticidal activity for all developed EO-NLCs against *A. gossypii* with values (% aphid survival) lower than <20%. In contrast, the formulations did not particularly affect the other target pests. Similarly, NLCs individually loaded with two EOs showed good insecticidal activity against *Culex pipiens* L. (Diptera: Culicidae) with an estimated LC₅₀ of 251.71 and 278.63 ppm and LC₉₀ of 637.52 and 825.55 for the fennel-NLC and green tea-NLC, respectively. Other authors highlighted the high biological activity of EOs in nano-emulsified formulations.^{32–35} As described by Kavallieratos *et al.* (2022),³⁶ a *Carlina acaulis* EO-based nano-emulsion (1000 ppm concentrated) exhibited good insecticidal activity (mortality rate of 93.9% and 98.9%) against *Tribolium*

castaneum (Herbst) and *Tribolium confusum* du Val (Coleoptera: Tenebrionidae), respectively. Similarly, Taktak *et al.* (2022)³⁷ developed different EO-based nano-emulsions (*i.e.*, cypress, lavender, lemon eucalyptus, and tea tree EO), which reported significant bioactivity with LC₅₀ values ranging from 57.10 to 180.70 mg L⁻¹ 48 hours after exposure.

In this context, this study aimed to develop a highly stable garlic EO-based nano-emulsion using the high-pressure microfluidization technique and to establish its biological activity on target and non-target organisms. The stability over time (up to 1 month) of the developed formulation was evaluated by dynamic light scattering analysis (DLS), and the particle size, the polydispersity index (PDI), and surface charge were assessed. The toxicological activity of the nano-emulsion against *A. gossypii* adults was investigated using two different methods, residual contact and topical application, and the lethal doses (LDs) were estimated. The estimated LDs against the target pest were used to evaluate adverse effects on non-target organisms, using ingestion toxicity against *Apis mellifera* L. (Hymenoptera: Apidae) and phytotoxicity trials on sweet pepper plants.

2. Materials and methods

2.1 Biological materials

The *A. gossypii* colony was reared for several generations on sweet pepper plants at the Entomology laboratories of the Department of Agriculture, University “Mediterranea” of Reggio Calabria, Reggio Calabria, Italy. The original colony was sourced from an organic citrus orchard (cv Clementine comune) located in Calabria (southern Italy). Rearing was carried out in BugDorm® cages under controlled environmental conditions (26 ± 1 °C, 65 ± 2% RH, and a 16 : 8 h light : dark photoperiod) to optimize colony development.

For toxicity trials, 1- to 2-day-old workers of *Apis mellifera ligustica* Spinola (Hymenoptera: Apidae) were collected from the experimental apiary of the same department. The bee colonies did not receive chemical treatments for at least three months prior to the experiments.

Sweet pepper plants used for both the aphids' rearing and the bioassays were cultivated from seeds in organic soil inside a climate-controlled chamber (25 ± 1 °C, 70 ± 5% RH, 14:10 h light:dark photoperiod). Plants that reached a height of 15–20 cm and developed 15 ± 1 leaves were used in the experiments.

2.2 Chemical characterization of garlic EO

Garlic EO was purchased from Esperis S.p.A. (Milan, Italy) and chemically analyzed using a gas chromatography-mass spectrometry (GC-MS) device, following the methods described by Giunti *et al.* (2019).³⁸ Briefly, a Thermo Fisher TRACE 1300 GC with a MEGA-5 capillary column (30 m × 0.25 mm; coating thickness = 0.25 μm) and a Thermo Fisher ISQ LT mass detector (ionization mode: EI; scan time: 1.00 s; scan mass range: 30–300 *m/z*) were used setting the injector and transfer line at 250 and 240 °C, respectively, and a



temperature ramp from 60 to 240 °C at 3 °C min⁻¹ (carrier gas: He 1 mL min⁻¹). The pure EO was diluted (1:10 v:v) in hexane (95%, Sigma Aldrich, Munich, Germany), and 0.2 µL was injected at a split ratio of 1:30. The identification of peaks was made using computer matching against commercial libraries (NIST 05, Wiley FFNSC, and ADAMS), comparing linear retention indices (LRIs). The LRIs were calculated using the formula of Van den Dool & Kratz (1963),³⁹ by comparing the retention times of the compounds to be identified with those of a standard mixture of alkanes (C8–C20 saturated alkane standard mixture, Supelco®, Bellefonte, PA, USA), which was analysed in GC-MS under identical operating conditions to the sample.^{39–44}

2.3 Garlic EO-based nano-emulsion development and physical characterization

The garlic EO-based nano-emulsion was developed using a top-down approach according to the method described by Modafferi *et al.* (2024).⁴⁵ In detail, garlic EO and Tween 80® (polyoxyethylene (20) sorbitan monooleate, Sigma Aldrich, Munich, Germany) (ratio 3:1 w:w) were mixed using a magnetic stirrer (5 min at 6000 rpm) to achieve a homogeneous oily phase. Double-distilled water was then added to the oily phase (ratio 4:1 v:w) and mixed for 5 min to obtain a raw emulsion (ratio 3:1:4 w:w:v for EO, Tween, and water, respectively). The obtained raw emulsion was homogenized using a high-pressure microfluidizer (HMP) device (LM20 Microfluidizer™ Processor, USA) with the pressure set at 30 000 PSI. The process was repeated five times, and to avoid EO degradation, the interaction chamber and heat exchanger were submerged in an ice bath. The obtained nano-emulsion was stored inside aluminum bottles and kept at 4 °C until the end of the experiments.

The developed garlic EO-based nano-emulsion was physically characterized using dynamic light scattering (DLS) analysis using a Zetasizer device (Zetasizer Nano, Malvern®). Specifically, the particle size (nm), polydispersity index (PDI), zeta potential (ζ-potential), and stability over time (*i.e.*, 24 hours, 1 week, and 1 month) were estimated. The analyses were conducted by diluting the developed formulation in double distilled water (ratio 1:200 v:v). For measurements, 1 mL and 0.75 mL of diluted nano-emulsion were used to assess the size and surface charge, respectively.

2.4 Biological activity against the target pest

The insecticidal activity of the developed garlic EO-based nano-emulsion was evaluated against *A. gossypii* adults using two different methods (*i.e.*, residual contact toxicity and topical application) in order to simulate real-world operating conditions. In both experiments, a total of six replicates of ten adult specimens were treated with different garlic EO-based nano-emulsion dilutions. Pure water and dimethoate (ROGOR® L40) water solution at label dose (0.1%) were used as negative (C-) and positive control (C+) treatments, respectively. Garlic EO-based nano-emulsion

dilutions were obtained by mixing the required amount of nano-emulsion in double-distilled water (w:v). All the experiments were carried out using the same rearing conditions (26 ± 1 °C, 65 ± 2% RH, and a photoperiod of 16:8 h L:D) (see section 2.1). In both trials, mortality was checked 48 and 72 hours after exposure. Insects were considered dead if they did not move or were unable to walk after stimulation with a fine brush.

Residual contact toxicity bioassays were conducted following the leaf-dip method. Specifically, circular sweet pepper leaves (Ø: 3.9 cm) were individually immersed for 15 s in different nano-emulsion dilutions (*i.e.*, 0.625, 0.93, 1.25, 1.87, and 2.5% of EO), C-, and C+, dried at room temperature (25 ± 1 °C), and placed inside ventilated plastic arenas (Ø: 4 cm; v: 50 mL). Subsequently, the specimens were gently placed on the treated surface, and the arenas were kept under the above-mentioned climatic conditions.

The topical application bioassay was performed using a professional hand sprayer (1 L Volpitech, Volpi®, Italy). A total of ten garlic EO-based nano-emulsion dilutions (*i.e.*, 0.111, 0.156, 0.233, 0.313, 0.465, 0.625, 0.93, 1.25, 1.87, and 2.5% of EO), C-, and C+ were tested. Infested sweet pepper plants were individually sprayed with the different treatments until run-off and left to dry under laboratory conditions (25 ± 1 °C). The treated insects were then carefully transferred with a fine brush to untreated leaf dishes' surface (Ø: 3.9 cm) and then incubated under the above conditions.

2.5 Toxicological evaluation towards honeybees

The impact of the developed garlic EO-based nano-emulsion towards honeybees was evaluated using the oral administration method as described by Medrzycki *et al.* (2013).⁴⁶ The ingestion route was preferred over other standard application methods (*i.e.*, topical and residual contact), because it was the most adequate to test acute toxicity against honeybees when considering *A. gossypii* as target pest species. Indeed, aphids can produce honeydew, a high-value food source foraged by honeybees, which can be contaminated by insecticide applications.

A total of five treatments were performed using the estimated LD₃₀, LD₅₀, and LD₉₀ (0.810, 1.079, and 2.171% of EO, respectively) obtained in residual contact toxicity bioassays (see Table 1), sucrose/water solutions (30% w/v) as negative controls (C-), and dimethoate (ROGOR® L40) at label dose (0.1%) as a positive control (C+). The desired LDs and C+ concentrations were obtained by diluting the required amount of nano-emulsion and active ingredient (a.i.), respectively, in a sucrose/water solution (30% w/v). The bees were collected and anesthetized with carbon dioxide. Then, ten specimens were gently placed inside ventilated cup-shaped hoarding arenas with a removable base, and two feeding devices containing the desired solution as described by Williams *et al.* (2013).⁴⁷ The trials were conducted under constant climatic conditions (25 ± 1 °C and 50 ± 5% RH). Each treatment was performed eight times, and the mortality



Table 1 Estimated lethal doses of the garlic EO-based nano-emulsion against *A. gossypii* 48 and 72 hours after the exposure in both methods. Values were considered significantly different if their 95% confidence limits did not overlap

LD ^a	Method	Time (h)	Estimate (EO dose%)	95% confidence limits	
				Lower bound	Upper bound
LD ₃₀	Residual ^b	48	0.997	0.878	1105
		72	0.810	0.699	0.907
	Topical ^c	48	0.148	0.073	0.218
		72	0.133	0.070	0.187
LD ₅₀	Residual	48	1.360	1.235	1.504
		72	1.079	0.970	1.189
	Topical	48	0.263	0.168	0.365
		72	0.212	0.140	0.283
LD ₉₀	Residual	48	2.904	2.459	3.705
		72	2.171	1.892	2.631
	Topical	48	1.086	0.724	2.308
		72	0.667	0.474	1.248

^a Lethal dose. ^b Residual contact toxicity method. ^c Topical application method.

of the specimens was recorded 4 hours after the treatment. The bees were considered dead if they were not able to fly or move after stimulation with a fine brush.

2.6 Phytotoxicity assessment on sweet pepper plants

The phytotoxic effects of the developed garlic EO-based nano-emulsion were evaluated on sweet pepper plants. In detail, plants, 15–20 cm in height, were individually treated using a hand sprayer until run-off and subsequently transferred to separate climate chambers (one per treatment). All plants were maintained under the same growth conditions (*i.e.*, 25 ± 1 °C, 70% RH, and a 14 : 10 h light : dark photoperiod). A total of four treatments were performed using the estimated LD₃₀, LD₅₀, and LD₉₀ (0.810, 1.079, and 2.171% of EO, respectively) obtained in the residual contact toxicity bioassays (see Table 1) 72 hours after *A. gossypii* exposure. Each treatment was replicated six times, and the phytotoxic effects were evaluated 1, 2, 5, and 10 days after plant treatment.

Phytotoxicity was evaluated through the calculation of the phytotoxicity index (Pi), a quantitative/qualitative index which includes both the number of damaged leaves and the severity of the damage. The Pi ranges from 0 (no damage) to 1 (dead leaves), and it has been calculated as described by Campolo *et al.* (2017):⁴⁸

$$Pi = \sum_{j=0}^n \left(\frac{DL_j}{TL} \times \frac{DC}{n-1} \right)$$

where DL is the number of damaged leaves for each damage severity class *j*; TL is the total number of leaves treated; DC is the damage severity class (0 = no damage; 1 = leaf surface with only chlorosis; 2 = leaves with evident necroses and 3 = dead leaves); *n* is the number of damage severity classes.

2.7 Data analysis

Data were analyzed using IBM® SPSS® Statistics 2v.23 (IBM Corp. Released 2015. Armonk, NY, USA). All data met the

assumptions required for parametric testing, including normality and homoscedasticity of variance (*p* > 0.05). Differences in the physical characteristics over time were analyzed using analysis of variance (ANOVA) with the particle size and polydispersity index (PDI) as dependent variables, and the times used as fixed factors. Multiple comparisons were conducted using Tukey's HSD *post hoc* test.

Mortality data in residual contact toxicity and topical bioassays against *A. gossypii* were corrected for control mortality using Abbott's formula.⁴⁹ The results obtained 48 and 72 h after the exposure in both bioassays fitted with the Probit model, and the LDs and their 95% confidence limits were estimated.

Differences among the different treatments on non-target organisms (*i.e.*, honeybees and sweet pepper plants) were analyzed using ANOVA, and multiple comparisons were assessed using the Duncan test.

3. Results

3.1 Garlic EO chemical composition

The chemical characterization of garlic EO is shown in SI 1. Specifically, twenty-four out of forty-four compounds were detected, accounting for 95.35 percent of the total calculated area. The EO was rich in sulfur compounds, with diallyl disulfide (27.41%), diallyl trisulfide (21.45%), diallyl sulfide (16.32%), diallyl tetrasulfide (11.20%), and 8-methyl-4,5,6,9-tetrathia-1,11-dodecadiene (7.52%) being the five most abundant detected compounds, representing more than 80% of the total area calculated.

3.2 Physical characterization of the garlic EO-based nano-emulsion

The physical characteristics of the developed garlic EO-based nano-emulsion showed optimal properties in terms of size, PDI, and surface charge. The size of the nano-emulsion increased over time (*F* = 7962.49; *df* = 2; *p* < 0.001) (Fig. 1A). Specifically, 24 hours after development, the nano-emulsion



exhibited very low particle size with a value of 86.03 ± 0.60 nm, while one week later the particle size increased to 130.3 ± 0.36 nm and after one month it was more than doubled (179 ± 1.4 nm) compared to the first measurement. Despite the increasing trend over time, the developed nano-emulsion remained in the nanometric range (<250 nm) until the end of the experiment. Regarding the PDI, the obtained garlic EO-based nano-emulsion showed a good particle size distribution with values always lower than 0.25 (Fig. 1B). Twenty-four hours after development, a very low PDI value (0.165 ± 0.001) was achieved. As observed for the particle size, the PDI increased over time too ($F = 352.83$; $df = 2$; $p < 0.001$), reaching values of 0.232 ± 0.006 and 0.227 ± 0.004 after 1 week and 1 month, respectively. Regarding the zeta potential results (Fig. 1C), the developed garlic EO-based nano-emulsion exhibited negative values ranging from -20.7 ± 0.31 to -28.1 ± 0.25 . Furthermore, statistical differences were recorded between the different observation times ($F = 676.02$; $df = 2$; $p < 0.001$).

3.3 Biological activity against *A. gossypii*

The developed garlic EO-based nano-emulsion exhibited high insecticidal activity against *A. gossypii* adults in both methods. Experimental data collected at 48 and 72 h after the exposure fitted with the Probit model in residual contact (48 h: $X^2 = 3.287$; $df = 3$; $p = 0.350$ and 72 h: $X^2 = 1.406$; $df = 3$; $p = 0.704$) and topical application (48 h: $X^2 = 3.073$; $df = 8$; $p = 0.930$ and 72 h: $X^2 = 4.018$; $df = 8$; $p = 0.856$), and the LDs and their 95% confidence limits were estimated (Fig. 2). In

both treatments (*i.e.*, residual contact and topical bioassays), no statistical differences were recorded between the exposure times (48 and 72 hours) within the same lethal dose, as their confidence limits overlapped. Conversely, statistical differences between the treatments were observed at both exposure times (*i.e.*, 48 and 72 hours) (Table 1). Specifically, topical application showed significantly higher toxicity than residual contact bioassays. Forty-eight hours after exposure, the estimated LDs (LD_{30} , LD_{50} , and LD_{90} of 0.148, 0.264, and 1.086% of EO, respectively) in the topical bioassay showed values lower than double those obtained in the residual contact toxicity bioassay (LD_{30} of 0.997, LD_{50} of 1.360, and LD_{90} of 2.904% of EO). A similar trend was observed 72 h after exposure, with LD_{30} , LD_{50} , and LD_{90} of 0.133, 0.212, and 0.667% of EO and 0.810, 1.079, and 2.171% of EO for the topical and residual contact toxicity bioassays, respectively. Complete LDs and their confidence limits in residual contact toxicity and topical application bioassays are shown in SI 2 and SI 3, respectively.

3.4 Biological activity towards honeybees

The biological activity of the developed garlic EO-based nano-emulsion toward honeybees is shown in Fig. 3. Honeybees were exposed to 0.810, 1.079, and 2.171% of EO, and the results highlighted the high insecticidal activity of the developed nano-emulsion, with mortality rates higher than ninety percent. No statistical differences were recorded among the tested garlic EO-based nano-emulsion doses and the C+ (dimethoate) group. Instead, the C- (sucrose water

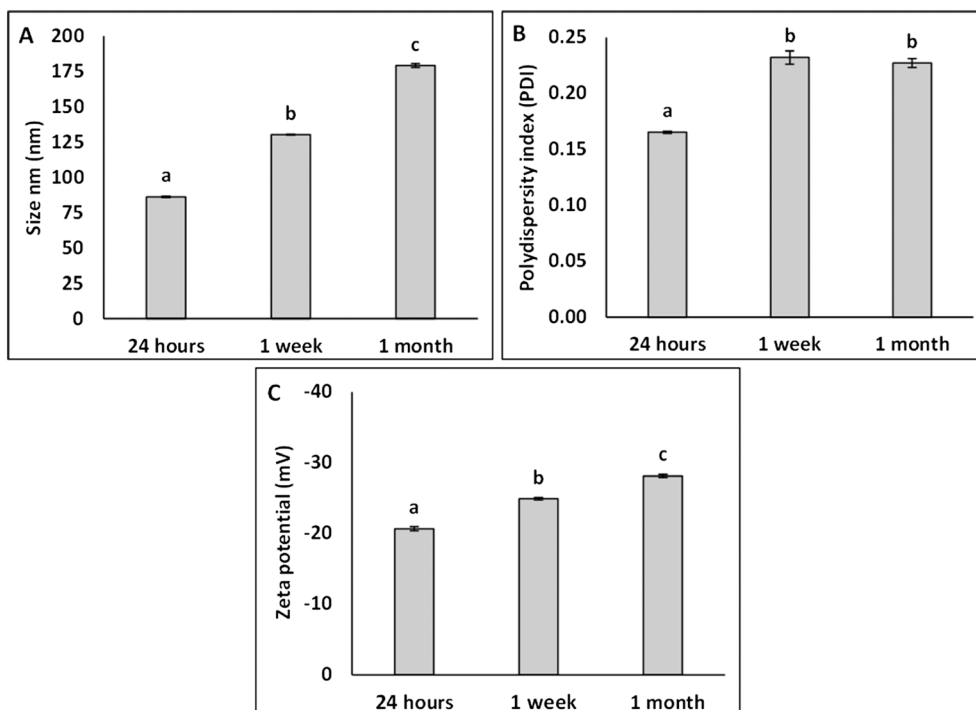


Fig. 1 Physical properties, size (A), polydispersity index (B), and zeta potential (C) of the developed garlic EO-based nano-emulsion. Values are means ($\pm$ standard deviation) of three replicates. Different letters indicate statistical differences over time (ANOVA, $p < 0.05$).



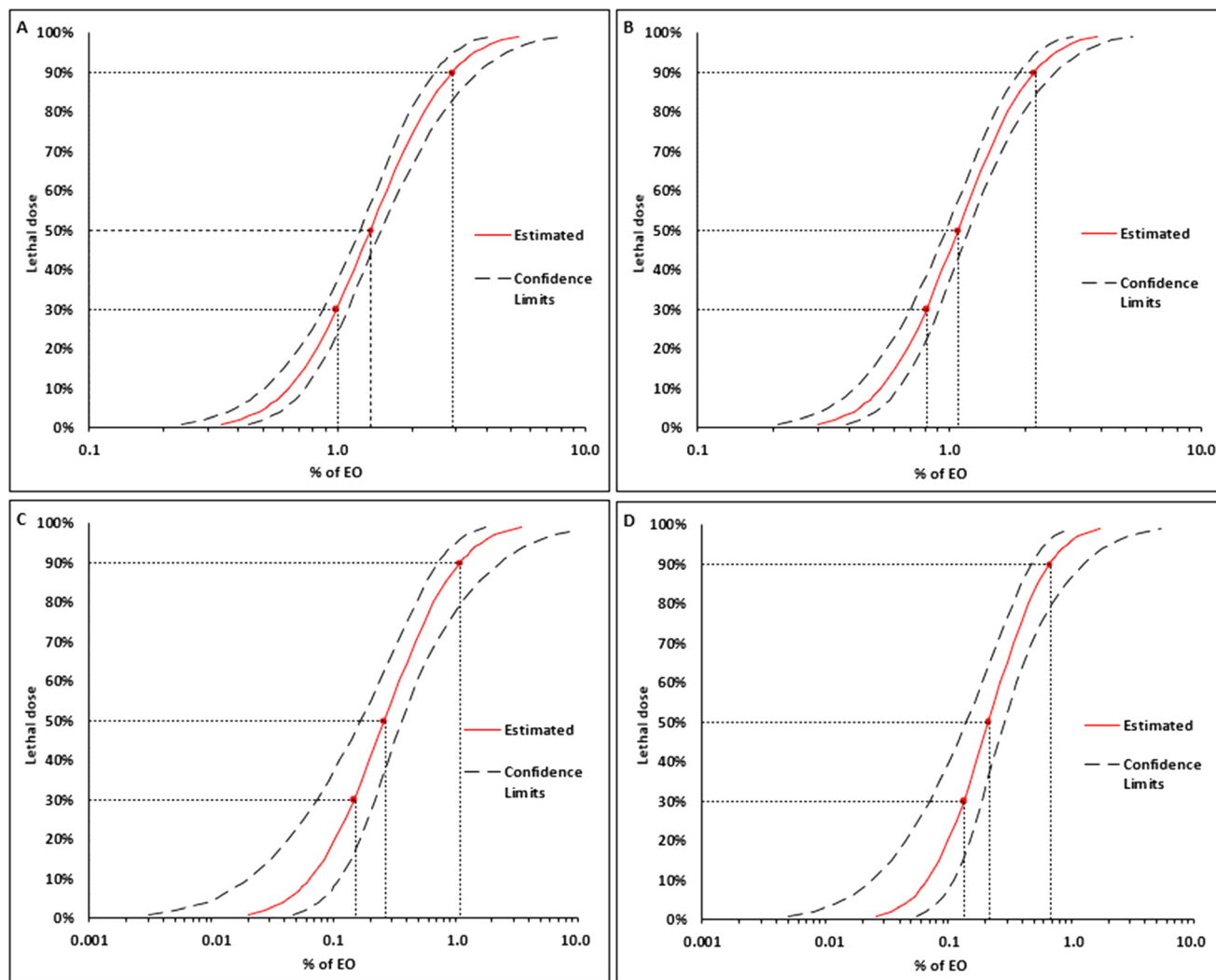


Fig. 2 Dose-response curves against the target pest: A) residual contact toxicity 48 hours after exposure; B) residual contact toxicity 72 hours after exposure; C) topical toxicity 48 hours after exposure; D) topical toxicity 72 hours after exposure. The x-axis is expressed in $\log_{10}$ scale.

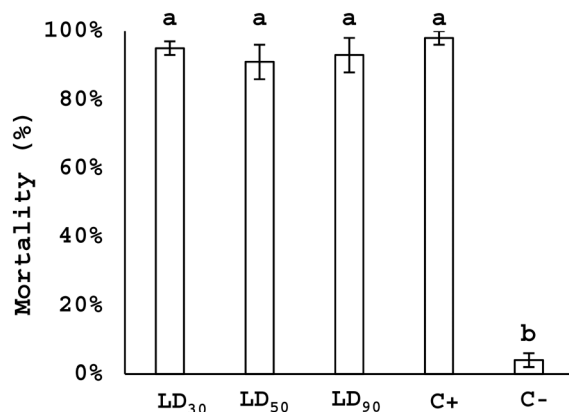


Fig. 3 Biological activity of garlic EO-based nano-emulsion doses (*i.e.*, residual contact LD_{30} , LD_{50} , and LD_{90} for aphids), positive control (C+), and negative control (C-) 4 hours after the honeybees' exposure. Values are means ($\pm$ standard error) of ten replicates. Different letters indicate statistical differences among the different treatments (ANOVA, $p < 0.05$).

solution 30%) group exhibited negligible mortality with statistically different outcomes ($F = 144.37$; $df = 4$; $p < 0.001$) compared to other treatments (*i.e.*, LD_{30} of 0.810, LD_{50} of 1.079, LD_{90} of 2.171% of EO, and C+).

3.5 Phytotoxicity to plants

The results of the phytotoxic bioassays on sweet pepper plants are shown in Fig. 4. Plant damage was estimated through the calculation of the phytotoxicity index (Pi), by accounting for both the severity and the ratio of injured leaves due to the treatment. In general, the Pi showed an increasing trend for all tested doses (*i.e.*, LD_{30} , LD_{50} , and LD_{90} for residual contact against aphids) up to 5 days after treatment, which decreased 10 days after the treatment with Pi values less than 0.05 for all the treatments. Conversely, no phytotoxicity was observed in the control groups ($Pi = 0$) until the end of the experiment. In detail, 1 day after the treatment, only LD_{90} showed a Pi value (0.072 ± 0.027)



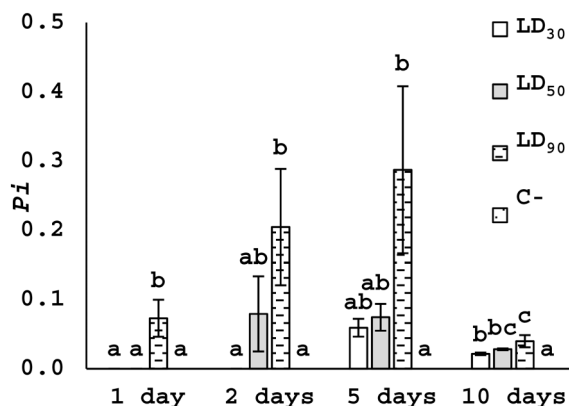


Fig. 4 Phytotoxicity index (Pi) on sweet pepper plants after different times of exposure (1, 2, 5, and 10 days) with different garlic EO-based nano-emulsion lethal doses (LD₃₀, LD₅₀, and LD₉₀) and the negative control (C-). Values are means ($\pm$ standard error) of six replicates. Different letters indicate statistical differences among the different treatments at the same time (ANOVA, $p < 0.05$).

statistically different ($F = 6.644$; $df = 3$; $p = 0.003$) compared to those of LD₃₀, LD₅₀, and C- which did not show phytotoxic effects ($Pi = 0$). Similarly, 2 days after the treatment, the LD₉₀ showed the highest Pi value (0.205 ± 0.084), which was significantly higher ($F = 3.911$; $df = 3$; $p = 0.025$) compared to LD₃₀ and C- treatment ($Pi = 0$), while the LD₅₀ group did not show statistical differences with other treatments ($Pi = 0.079 \pm 0.054$). After 5 days, LD₉₀ treatment provoked a Pi value (0.287 ± 0.012) more than double compared to Pi values of LD₃₀ and LD₅₀ groups (0.059 ± 0.013 and 0.074 ± 0.019 respectively); however, statistical differences were recorded only between LD₉₀ and C- (Pi values of 0.287 ± 0.012 and 0, respectively) ($F = 3.207$; $df = 3$; $p = 0.045$). At the end of the experiments (10 days), very low Pi values ($Pi < 0.05$) were recorded in all the treatments, and statistical differences ($F = 12.77$; $df = 3$; $p < 0.001$) were noted among C-, LD₃₀, and LD₉₀ groups (0, 0.021 ± 0.002 , and 0.039 ± 0.009 Pi , respectively).

4. Discussion

The analysis of the chemical composition revealed that garlic EO was rich in sulfur compounds (95.35%), with diallyl disulfide (27.41%) and diallyl trisulfide (21.45%) being the most abundant compounds detected. These findings are consistent with our previous study, which also identified these two compounds as the major constituents of garlic EO.^{50,51} Moreover, other studies have reported that different varieties of garlic were similarly rich in these organosulfur compounds.^{52,53} However, it should be noted that the composition of EOs can be influenced by several factors, including the drying method, extraction process, and analytical techniques used.^{54,55}

In this study, a highly stable garlic EO-based nano-emulsion with a high amount of a.i. (15% of EO) was obtained using an HPM process. Generally, nano-emulsions

can be obtained using different approaches, like the one used in this study (HPM) or sonication and high-pressure homogenization (HPH).^{56,57} Among these, HPM is the most efficient due to the high impact forces and the ability to use it in large-scale production.^{58,59} Furthermore, the HPM process was well known to produce EO-based nano-emulsions with particle sizes ranging in the nanoscale (<250 nm) and low PDI values (<0.3).^{60,61} In this context, the proposed garlic EO-based nano-emulsion showed optimal physical characteristics comparable with those obtained by other authors who used the same process. As described by Xing *et al.* (2024),⁶² the HPM process was able to produce different cinnamon EO-based nano-emulsions with particle size values (ranging from 86.84 ± 0.53 nm to 107.50 ± 1.56 nm) comparable to those obtained from our nano-emulsion 24 hours after preparation (86.03 ± 0.60 nm). Similarly, Dimak *et al.* (2024),⁶³ developed a peppermint EO-based nano-emulsion with a very fine droplet size (69.8 nm) and PDI values slightly higher than those reported for our nano-emulsions 1 month after formulation (0.277 and 0.227, respectively). Other authors, using the HPM method, developed different EO-based nano-emulsions (*i.e.*, thyme, lemongrass, cinnamon, peppermint, and clove EOs), all ranging in the nanoscale for at least 1 month, with particle size values (<160 nm for all nano-emulsions) lower than those recorded for our 1-month-old nano-emulsions (179 ± 1.4 nm).⁶⁴ However, the amount of EO used in these nano-emulsions was very low compared to the one used in this study (2.5 vs. 15%, respectively). The amount of EO loaded in the nano-formulations for pesticide applications should be maximized, as this is a key aspect of their effectiveness and commercial viability. Indeed, a low concentration of the a.i. may be ineffective and require large volumes of product to cover the wide areas of crop fields, which leads to storage, transport, and application challenges.²⁹ Another important feature of the garlic EO-based nano-emulsion developed in this study is the relatively low amount of co-formulant (EO: surfactant ratio 3:1) employed to achieve stabilization. It is well known that high amounts of co-formulants can promote the development of EO-based nano-emulsions with very small droplet sizes and PDI values close to zero. Nevertheless, excessive use of these substances negatively affects the plants, leading to plant growth and cell membrane permeability alterations, increased contaminant uptake, and damage to vegetable tissues. For this reason, minimizing the amounts of co-formulants used is essential to preserve the compatibility of the formulation with crop plants.^{65–67} The ζ -potential is one of the most important parameters for assessing the colloidal stability of nano-emulsions. Generally, ζ -potential values around ± 30 mV are considered optimal for ensuring system stability.^{68,69} Our results fall within this range with ζ -potential values from 20.7 ± 0.31 to 28.1 ± 0.25 , confirming high colloidal stability. Moreover, the use of non-ionic surfactants (*i.e.*, Tween 80) significantly contributes to stability through a combination of electrostatic and steric repulsion. Under these conditions, nano-emulsions may



remain stable due to ζ -potential values of approximately ± 20 mV, maintaining droplet sizes in the nanoscale range.^{50,70,71}

In this study, the developed garlic EO-based nano-emulsion showed high insecticidal activity against *A. gossypii* adults with low estimated LD values (LD₅₀ and LD₉₀ of 1.079 and 2.171% of EO, respectively) in residual contact application and LD values more than halved in topical application (LD₅₀ of 0.212% of EO and LD₉₀ of 0.667% of EO) 72 h after exposure. To the best of our knowledge, this is the first study that proves the high insecticidal activity of garlic EO formulated in nano-emulsion against the cotton aphid. However, the biological activity of other EO-based nano-emulsions against this pest was investigated in previous studies.^{72,73} As an example, Laudani *et al.* (2022)⁷⁴ developed a *Citrus sinensis* EO-based nano-emulsion and evaluated its biological activity against this pest through a topical application method. Although this nano-emulsion showed good insecticidal activity after 48 h, the estimated LD₅₀ and LD₉₀ (1.48 and 2.86% of EO, respectively) were greater compared to the values obtained in the present study after the same exposure time (LD₅₀ of 0.263% of EO and LD₉₀ of 1.086% of EO). This suggests the higher insecticidal efficacy of the garlic EO-based nano-emulsion compared to the one formulated with a different a.i., *C. sinensis* EO. Similarly, two different natural pyrethrin-based nano-emulsions were able to reduce the insect population by approximately 90% up to 72 hours after exposure when topically applied.⁷⁵ The efficacy of EO-based nano-emulsions was well-proven against other aphid species. A spearmint EO-based nano-emulsion exhibited good insecticidal activity with an estimated LC₅₀ of 2.87–2.81 mg mL⁻¹ and an acetylcholinesterase inhibitory activity (IC)₅₀ of 1.66–5.34 mg mL⁻¹ against *Rhopalosiphum maidis* (Fitch) and *Sitobion avenae* F. (Hemiptera: Aphididae), respectively.⁷⁶ Furthermore, other authors reported the efficacy of several EO-based nano-emulsions against *Aphis craccivora* Koch and *Aphis fabae* Scop. (Hemiptera: Aphididae).^{77–80}

Despite growing interest in botanical nano-insecticides, their ecological impacts are poorly investigated. Most studies focus on metallic or synthetic nano-formulations,^{81,82} highlighting the need for research on these formulations. However, many concerns for synthetic nanomaterials may not apply to botanical nano-insecticides, whose natural origin generally promotes rapid biodegradation, which in turn contributes to their selectivity toward non-target organisms (*e.g.*, pollinators, natural antagonists, and aquatic invertebrates) and facilitates the translation of promising laboratory results into practical field applications.^{29,83} Nevertheless, only a few studies investigated the biological activity of these formulations on non-target organisms, and their fate in the environment is not easy to foresee. As shown by Mazzara *et al.* (2023),⁸⁴ the estimated LC₉₀ (207.2 ppm) of a *Cannabis sativa* L. EO-based nano-emulsion against *Culex quinquefasciatus* Say (Diptera: Culicidae) did not significantly affect the survival (mortality <16% 48 h after the exposure) of an aquatic microcrustacean (*i.e.*, *Daphnia magna*). Similarly, a

Schinus terebinthifolius Raddi EO-based nano-emulsion exhibited high insecticidal activity against *C. pipiens* with an estimated LC₉₀ of 13.2 and 11.3 μ L L⁻¹ on larvae and adults, respectively. However, toxicological trials toward non-target larvorous fish such as *Gambusia affinis* proved no significant effect on the survival (LC₅₀ and LC₉₀ of 3042.7 and 5614.7 μ L mL⁻¹), longevity, and swimming activity of the non-target species; in addition, up to a concentration of 200 mg kg⁻¹, no mortality was observed also on *Eisenia fetida*.⁸⁵

Concerning pollinators, a *Persea venosa* Nees & Mart EO nano-emulsion did not cause any mortality toward bees.⁸⁶ Nevertheless, the effects of these environmentally friendly formulations depend on several factors, including the type of EO and non-target insect, the application rate, the dose, and the route of exposure.^{27,87} In this regard, previous research demonstrated that the estimated LD₅₀ and LD₉₀ for the pest *Planococcus citri* Risso (Hemiptera: Pseudococcidae) of different garlic EO-based nano-emulsions had no impact on the survival of honeybees through topical application (100% survival), while the positive control (dimethoate at 0.1%) led to complete mortality.⁵⁰ Nevertheless, pollinators and parasitoids may come into contact with insecticide residues also when foraging for food sources on flowers, as well as on honeydew produced by aphid species. In this regard, in this study the acute toxicity of the garlic nano-emulsion was tested in oral administration trials, to account for this kind of exposure. In contrast to topical toxicity trials,⁵⁰ here the toxicological results on honeybees highlighted that the garlic EO-based nano-emulsion was highly toxic when administered *via* ingestion, resulting in 100% mortality. A comparable outcome was reported by Modafferi *et al.* (2025),⁸⁸ who estimated the LDs (LD₃₀, LD₅₀, and LD₉₀ of 0.69, 0.96, and 2.18% of EO, respectively) against the target pest and investigated the toxicological impact against a non-target parasitoid, *Aganispis daci* (Weld) (Hymenoptera: Figitidae), emphasizing the high insecticidal activity (100% mortality) of ingestion administration at all the tested doses. These findings indicate that the selectivity of botanical nano-formulations, particularly those based on garlic EO, requires further investigation to fully assess their bioactivity against non-target insects, including honeybees. When comparing the results from topical and oral trials, it is clear that ingestion poses a significantly higher risk to honeybee survival, whereas contact exposure appears to be safe. This suggests that the application of the garlic EO-based nano-emulsion should be avoided during crop flowering, as food resources for natural antagonists and pollinators (*i.e.*, pollen and nectar) are particularly abundant at this stage, potentially leading to greater ingestion of these harmful substances. Overall, the risks associated with insecticide use during this critical crop phase are quite well acknowledged, and its use at flowering is inhibited.^{89–91} Furthermore, the presence of massive alternative food sources, as honeydew produced by aphid species, may also trigger serious damage to honeybees, as well as to several other non-target species foraging on it.



Lastly, concerning the outcomes observed in sweet pepper plants after exposure to the developed garlic EO-based nano-emulsion, low phytotoxic effects were detected, and only the estimated LD₉₀ (2.171% of EO) resulted in a Pi value of about 0.3 up to 5 days, which decreased to no significant effect 10 days after treatment. Differently, a comparable nano-emulsion containing 3% of the a.i. resulted in very low Pi values (0.13 ± 0.1), although the treated plants showed less fruits per plant (<3) compared to the untreated control group (4.2 ± 0.4).⁹² However, as previously outlined, the adverse effects depend on several factors, including the plant species. For instance, Ricupero *et al.* (2022)⁹³ demonstrated that a garlic nano-emulsion did not exhibit phytotoxic effects on tomato plants. A similar outcome was observed in citrus plants treated with a comparable nano-emulsion. In addition, garlic treatment enhanced the natural plant defense system through the expression of different genes involved in salicylic and jasmonic pathways.⁵¹

5. Conclusion

This study highlighted the potential effectiveness of a novel green nano-pesticide as an alternative to conventional formulations for *A. gossypii* control. The proposed garlic EO-based nano-emulsion obtained through microfluidization showed optimal physical properties with nanoscale particle size (<200 nm), low PDI values (<0.25), and negative surface charge up to 1 month after development. The result obtained in toxicological bioassays against adults *A. gossypii* showed a high mortality rate in both methods (*i.e.*, residual contact and topical application), highlighting the good efficacy of the developed nano-emulsion in the target pest control. Furthermore, minimal phytotoxic effects on pepper plants (Pi < 0.1) until the end of the experiment were highlighted. On the other hand, the garlic nano-emulsion exhibited a high mortality rate (100%) towards honeybees by ingestion. These results underline that, although this nano-emulsion is highly effective against the target pest, the adverse effects on non-target organisms need to be carefully considered to minimize their impact under open field conditions. Furthermore, our results shed light on the need for further investigations on the bioactivity of green nano-insecticides on key non-target organisms, including pollinators, before their application in agroecosystems.

Author contributions

AM, GG, and OC: conceptualization; AM, GG, and OC: formal analysis; VP: funding acquisition; AM, ML, MP, and RC: investigation; IL and PF: validation. AM: writing the original draft. All authors reviewed, edited and approved the manuscript.

Conflicts of interest

There are no conflicts to declare.

Data availability

Data for this article, including physical characterization of the nano-emulsion and toxicological trials, are available at Mendeley Data at <https://doi.org/10.17632/tbb6ggcmgs.1>.

Chemical characterization of garlic essential oil by GC-MS has been included as part of the supplementary information (SI). Supplementary information is available. See DOI: <https://doi.org/10.1039/d5en00498e>.

Acknowledgements

Maria Pineda was supported by the project "Technologies for climate change adaptation and quality of life improvement" - EI TECH4YOU - Spoke 3 - (Smart technologies for sustainable agrifood chain and forestry), Goal 3.1 (Agriculture and livestock smart farming), PP 3.1.1 (Targeting precision farming applications to agroecological intensification). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

References

- 1 L. Wang, S. Zhang, J. Y. Luo, C. Y. Wang, L. M. Lv and X. Z. Zhu, *et al.*, Identification of *Aphis gossypii* glover (Hemiptera: Aphididae) biotypes from different host plants in North China, *PLoS One*, 2016, **11**(1), e0146345, DOI: [10.1371/journal.pone.0146345](https://doi.org/10.1371/journal.pone.0146345).
- 2 M. D. Michelotto and A. C. Busoli, Eficiência de ninfas e adultos de *Aphis gossypii* Glov. na transmissão do vírus do mosaico das nervuras do algodoeiro, *Bragantia*, 2003, **62**, 255–259, DOI: [10.1590/S0006-87052003000200010](https://doi.org/10.1590/S0006-87052003000200010).
- 3 J. Liu, C. Wang, N. Desneux and Y. Lu, Impact of temperature on survival rate, fecundity, and feeding behavior of two aphids, *Aphis gossypii* and *Acyrtosiphon gossypii*, when reared on cotton, *Insects*, 2021, **12**(6), 565, DOI: [10.3390/insects12060565](https://doi.org/10.3390/insects12060565).
- 4 A. Urbaneja, T. G. Grout, S. Gravena, F. Wu, Y. Cen and P. A. Stansly, Citrus pests in a global world, *The Genus Citrus*, 2020, DOI: [10.1016/B978-0-12-812163-4.00016-4](https://doi.org/10.1016/B978-0-12-812163-4.00016-4).
- 5 N. M. El-Shourbagy, S. M. Farag, M. A. M. Moustafa, L. A. Al-Shuraym, S. Sayed and O. H. Zyaan, Biochemical and insecticidal efficacy of clove and basil essential oils and two photosensitizers and their combinations on *Aphis gossypii* glover (Hemiptera: Aphididae), *BioScience*, 2023, **39**, e39100, DOI: [10.14393/BJv39n0a2023-69129](https://doi.org/10.14393/BJv39n0a2023-69129).
- 6 O. Campolo, G. Giunti, A. Russo, V. Palmeri and L. Zappalà, Essential oils in stored product insect pest control, *J. Food Qual.*, 2018, (1), 6906105, DOI: [10.1155/2018/6906105](https://doi.org/10.1155/2018/6906105).
- 7 R. Pavela and G. Benelli, Essential oils as ecofriendly biopesticides? Challenges and constraints, *Trends Plant Sci.*, 2016, **21**, 1000–1007, DOI: [10.1016/j.tplants.2016.10.005](https://doi.org/10.1016/j.tplants.2016.10.005).
- 8 E. Assadpour, A. Can Karaça, M. Fasamanesh, S. A. Mahdavi, M. Shariat-Alavi and J. Feng, *et al.*, Application of essential oils as natural biopesticides; recent advances, *Crit. Rev. Food Sci. Nutr.*, 2024, **64**(19), 6477–6497, DOI: [10.1080/10408398.2023.2170317](https://doi.org/10.1080/10408398.2023.2170317).



- 9 P. Czerniewicz, G. Chrzanowski, I. Sprawka and H. Sytykiewicz, Aphicidal activity of selected Asteraceae essential oils and their effect on enzyme activities of the green peach aphid, *Myzus persicae* (Sulzer), *Pestic. Biochem. Physiol.*, 2018, **145**, 84–92, DOI: [10.1016/j.pestbp.2018.01.010](#).
- 10 A. Kasmi, M. Hammami, E. G. Raoelison, M. Abderrabba, J. Bouajila and C. Ducamp, Chemical composition and behavioral effects of five plant essential oils on the green pea aphid *Acyrtosiphon pisum* (Harris) (Homoptera: Aphididae), *Chem. Biodiversity*, 2017, **14**(5), e1600464, DOI: [10.1002/cbdv.201600464](#).
- 11 L. L. A. Bezerra, F. R. Azevedo, W. S. Evangelista-Júnior, F. J. Paula-Filho, D. M. A. F. Navarro and E. F. Santos, Efficiency of essential oils in the control of the black bean aphid *Aphis craccivora* Koch (Hemiptera: Aphididae), *Braz. J. Biol.*, 2023, **83**, e275069, DOI: [10.1590/1519-6984.275069](#).
- 12 F. Behi, O. Bachrouch and S. Boukhris-Bouhachem, Insecticidal activities of *Mentha pulegium* L., and *Pistacia lentiscus* L., essential oils against two citrus aphids *Aphis spiraecola* Patch and *Aphis gossypii* glover, *J. Essent. Oil Bear. Plants*, 2019, **22**(2), 516–525, DOI: [10.1080/0972060X.2019.1611483](#).
- 13 S. Sayed, M. M. Soliman, S. Al-Otaibi, M. M. Hassan, S. A. Elarnnaouty and S. M. Abozeid, *et al.*, Toxicity, deterrent and repellent activities of four essential oils on *Aphis punicae* (Hemiptera: Aphididae), *Plants*, 2022, **11**(3), 463, DOI: [10.3390/plants11030463](#).
- 14 Z. Koorki, S. Shahidi-Noghabi, K. Mahdian and M. Pirmaoradi, Chemical composition and insecticidal properties of several plant essential oils on the melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), *J. Essent. Oil Bear. Plants*, 2018, **21**(2), 420–429, DOI: [10.1080/0972060X.2018.1435308](#).
- 15 X. Wang, Y. Zhang, H. Yuan and Y. Lu, Effects of seven plant essential oils on the growth, development and feeding behavior of the wingless *Aphis gossypii* Glover, *Plants*, 2024, **13**, 916, DOI: [10.3390/plants13070916](#).
- 16 F. Albouchi, N. Ghazouani, R. Souissi, M. Abderrabba and S. Boukhris-Bouhachem, Aphidicidal activities of *Melaleuca styphelioides* Sm. essential oils on three citrus aphids: *Aphis gossypii* Glover; *Aphis spiraecola* Patch and *Myzus persicae* (Sulzer), *S. Afr. J. Bot.*, 2018, **117**, 149–154, DOI: [10.1016/j.sajb.2018.05.005](#).
- 17 R. Abdullah, W. Aziz and N. Ghanim, Toxicity and biochemical impacts of garlic and camphor essential oils against *Aphis gossypii* and *Gynaikothrips ficorum* compared to chitosan and mineral oil, *J. Plant Prot. & Pathol.*, 2024, **15**, 173–181, DOI: [10.21608/jppp.2024.290485.1235](#).
- 18 K. Aiad, Utilizing of plant extract garlic oil against *Aphis Gossypii* and *Tetranychus Urticae*, *Ann. Agric. Sci.*, 2019, **57**(1), 151–154, DOI: [10.21608/assjm.2019.42229](#).
- 19 M. Hori, Settling inhibition and insecticidal activity of garlic and onion oils against *Myzus persicae* (SULZER) (Homoptera: Aphididae), *Appl. Entomol. Zool.*, 1996, **31**(4), 605–612, DOI: [10.1303/aez.31.605](#).
- 20 K. M. Mousa, I. A. Khodeir, T. N. El Dakhakhni and A. E. Youssef, Effect of garlic and eucalyptus oils in comparison to organophosphate insecticides against some piercing-sucking faba bean insect pests and natural enemies populations, *Egypt. Acad. J. Biol. Sci., F Toxicol.*, 2013, **5**(2), 21–27, DOI: [10.21608/eajbsf.2013.17266](#).
- 21 O. Koul, S. Walia and G. S. Dhaliwal, Essential oils as green pesticides: Potential and constraints, *Biopestic. Int.*, 2008, **4**(1), 63–84.
- 22 H. A. H. Said-Al Ahl, W. Hikal, K. Tkachenko, H. A. H. Said-Al Ahl and H. A. H. Said-Al Ahl, *et al.*, Essential oils with potential as insecticidal agents: A review, *International Journal of Environmental Planning and Management*, 2017, **3**(4), 23–33.
- 23 M. Sessa and F. Donsi, Nanoemulsion-based delivery systems, *Microencapsulation and microspheres for food applications*, 2015, pp. 79–94, DOI: [10.1016/B978-0-12-800350-3.00007-8](#).
- 24 S. S. Mukhopadhyay, Nanotechnology in agriculture: Prospects and constraints, *Nanotechnol., Sci. Appl.*, 2014, **7**, 63–71, DOI: [10.2147/NSA.S39409](#).
- 25 A. Gupta, F. Rayeen, R. Mishra, M. Tripathi and N. Pathak, Nanotechnology applications in sustainable agriculture: An emerging eco-friendly approach, *Plant Nano Biol.*, 2023, **4**, 100033, DOI: [10.1016/j.plana.2023.100033](#).
- 26 N. Bidyarani, J. Jaiswal and U. Kumar, Botanicals-based nanoformulations for the management of insect-pests, in *Nanophytopathology*, 2023, pp. 173–184.
- 27 R. T. Gahukar and R. K. Das, Plant-derived nanopesticides for agricultural pest control: challenges and prospects, *Nanotechnol. Environ. Eng.*, 2020, **5**, 1–9, DOI: [10.1007/s41204-020-0066-2](#).
- 28 F. Esmaili, A. Sanei-Dehkordi, F. Amoozegar and M. Osanloo, A review on the use of essential oil-based nanoformulations in control of mosquitoes, *Biointerface Res. Appl. Chem.*, 2021, **11**(5), 12516–12529, DOI: [10.33263/BRIAC115.1251612529](#).
- 29 A. Modafferi, G. Giunti, G. Benelli and O. Campolo, Ecological costs of botanical nano-insecticides, *Curr. Opin. Environ. Sci. Health*, 2024, 100579, DOI: [10.1016/j.coesh.2024.100579](#).
- 30 R. Zannat, M. Rahman and M. Afroz, Application of nanotechnology in insect pest management: A review, *SAARC J. Agric.*, 2022, **19**(2), 1–11, DOI: [10.3329/sja.v19i2.57668](#).
- 31 S. Tortorici, C. Cimino, M. Ricupero, T. Musumeci, A. Biondi and G. Siscaro, *et al.*, Nanostructured lipid carriers of essential oils as potential tools for the sustainable control of insect pests, *Ind. Crops Prod.*, 2022, **181**, 114766, DOI: [10.1016/j.indcrop.2022.114766](#).
- 32 F. N. Gharsan, Bioactivity of plant nanoemulsions against stored-product insects (order coleoptera): a review¹, *J. Entomol. Sci.*, 2024, **59**, 355–365.
- 33 L. Pavoni, R. Pavela, M. Cespi, G. Bonacucina, F. Maggi and V. Zeni, *et al.*, Green micro-and nanoemulsions for managing parasites, vectors and pests, *Nanomaterials*, 2019, **9**(9), 1285, DOI: [10.3390/nano9091285](#).
- 34 K. S. AnnaDurai, N. Chandrasekaran, S. Velraja, G. S. Hikku and V. D. Parvathi, Essential oil nanoemulsion: An emerging



- eco-friendly strategy towards mosquito control, *Acta Trop.*, 2024, **257**, 107290, DOI: [10.1016/j.actatropica.2024.107290](https://doi.org/10.1016/j.actatropica.2024.107290).
- 35 S. Subramanya, K. Sumanth, P. K. Gupta, V. Chayapathy, E. Keshamma and K. Murugan, Formulation of green nanoemulsions for controlling agriculture insects, *Bio-Based Nanoemulsions for Agri-Food Applications*, 2022, DOI: [10.1016/B978-0-323-89846-1.00002-4](https://doi.org/10.1016/B978-0-323-89846-1.00002-4).
 - 36 N. G. Kavallieratos, E. P. Nika, A. Skourti, M. C. Boukouvala, C. T. Ntalaka and F. Maggi, *et al.*, *Carlina acaulis* essential oil nanoemulsion as a new grain protectant against different developmental stages of three stored-product beetles, *Pest Manage. Sci.*, 2022, **78**(6), 2434–2442, DOI: [10.1002/ps.6877](https://doi.org/10.1002/ps.6877).
 - 37 N. E. M. Taktak, M. E. I. Badawy, O. M. Awad and N. E. Abou El-Ela, Nanoemulsions containing some plant essential oils as promising formulations against *Culex pipiens* (L.) larvae and their biochemical studies, *Pestic. Biochem. Physiol.*, 2022, **185**, 105151, DOI: [10.1016/j.pestbp.2022.105151](https://doi.org/10.1016/j.pestbp.2022.105151).
 - 38 G. Giunti, D. Palermo, F. Laudani, G. M. Algeri, O. Campolo and V. Palmeri, Repellence and acute toxicity of a nano-emulsion of sweet orange essential oil toward two major stored grain insect pests, *Ind. Crops Prod.*, 2019, **142**, 111869, DOI: [10.1016/j.indcrop.2019.111869](https://doi.org/10.1016/j.indcrop.2019.111869).
 - 39 H. van Den Dool and P. D. Kratz, A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography, *J. Chromatogr.*, 1963, **11**, 463–471.
 - 40 R. P. Adams, *Identification of essential oil components by gas chromatography/mass spectrometry*, Texensis Publishing, Gruver, TX USA, 5th edn, 2017, pp. 46–52.
 - 41 N. W. Davies, Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and Carbowax 20M phases, *J. Chromatogr.*, 1990, **503**, 1–24.
 - 42 W. Jennings, *Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography*, Elsevier, 2012.
 - 43 Y. Masada, *Analysis of essential oils by gas chromatography and mass spectrometry*, 1976.
 - 44 F. McLafferty and D. Stauffer, *The Wiley/NBS registry of mass spectral data*, 1989.
 - 45 A. Modafferi, M. Ricupero, G. Mostacchio, I. Latella, L. Zappalà and V. Palmeri, *et al.*, Bioactivity of *Allium sativum* essential oil-based nano-emulsion against *Planococcus citri* and its predator *Cryptolaemus montrouzieri*, *Ind. Crops Prod.*, 2024, **208**, 117837, DOI: [10.1016/j.indcrop.2023.117837](https://doi.org/10.1016/j.indcrop.2023.117837).
 - 46 P. Medrzycki, H. Giffard, P. Aupinel, L. P. Belzunces, M. P. Chauzat and C. Claßen, *et al.*, Standard methods for toxicology research in *Apis mellifera*, *J. Apic. Res.*, 2013, **52**, 111869, DOI: [10.3896/IBRA.1.52.4.14](https://doi.org/10.3896/IBRA.1.52.4.14).
 - 47 G. R. Williams, C. Alaux, C. Costa, T. Csáki, V. Doublet and D. Eisenhardt, *et al.*, Standard methods for maintaining adult *Apis mellifera* in cages under in vitro laboratory conditions, *J. Apic. Res.*, 2013, **52**(1), 1–36, DOI: [10.3896/IBRA.1.52.1.04](https://doi.org/10.3896/IBRA.1.52.1.04).
 - 48 O. Campolo, A. Cherif, M. Ricupero, G. Siscaro, K. Grissa-Lebdi and A. Russo, *et al.*, Citrus peel essential oil nanoformulations to control the tomato borer, *Tuta absoluta*: Chemical properties and biological activity, *Sci. Rep.*, 2017, **7**(1), 13036, DOI: [10.1038/s41598-017-13413-0](https://doi.org/10.1038/s41598-017-13413-0).
 - 49 W. S. Abbott, A method for computing the effectiveness of an insecticide, *J. Econ. Entomol.*, 1925, **18**(2), 265–267.
 - 50 A. Modafferi, G. Giunti, A. Urbaneja, F. Laudani, I. Latella and M. Pérez-Hedo, *et al.*, High-energy emulsification of *Allium sativum* essential oil boosts insecticidal activity against *Planococcus citri* with no risk to honeybees, *J. Pest Sci.*, 2024, 1–12, DOI: [10.1007/s10340-024-01800-2](https://doi.org/10.1007/s10340-024-01800-2).
 - 51 A. Modafferi, A. Urbaneja, C. M. Aure, F. Laudani, V. Palmeri and G. Giunti, *et al.*, Green pest control strategies: Essential oil-based nano-emulsions for *Delottococcus aberiae* management, *J. Pest Sci.*, 2025, **98**, 1263–1275, DOI: [10.1007/s10340-025-01914-1](https://doi.org/10.1007/s10340-025-01914-1).
 - 52 O. El Faqer, S. Rais, Z. Ouadghiri, A. El Faqer, Z. Benchama and A. El Ouaddari, *et al.*, Physicochemical properties, GC–MS profiling, and antibacterial potential of *Allium sativum* essential oil: In vitro and in silico approaches, *Sci. Afr.*, 2024, **26**, 02484, DOI: [10.1016/j.sciaf.2024.e02484](https://doi.org/10.1016/j.sciaf.2024.e02484).
 - 53 S. Sommano, N. Saratan, R. Suksathan and T. Pusadee, Chemical composition and comparison of genetic variation of commonly available Thai garlic used as food supplement, *J. Appl. Bot. Food Qual.*, 2016, **89**, 235–242, DOI: [10.5073/JABFQ.2016.089.030](https://doi.org/10.5073/JABFQ.2016.089.030).
 - 54 P. Satyal, J. D. Craft, N. S. Dosoky and W. N. Setzer, The Chemical compositions of the volatile oils of garlic (*Allium sativum*) and wild garlic (*Allium vineale*), *Foods*, 2017, **6**, 63, DOI: [10.3390/foods6080063](https://doi.org/10.3390/foods6080063).
 - 55 C. Condurso, F. Cincotta, G. Tripodi, M. Merlino and A. Verzera, Influence of drying technologies on the aroma of Sicilian red garlic, *LWT–Food Sci. Technol.*, 2019, **104**, 180–185, DOI: [10.1016/j.lwt.2019.01.026](https://doi.org/10.1016/j.lwt.2019.01.026).
 - 56 F. Donsì and G. Ferrari, Essential oil nanoemulsions as antimicrobial agents in food, *J. Biotechnol.*, 2016, **233**, 106–120, DOI: [10.1016/j.jbiotec.2016.07.005](https://doi.org/10.1016/j.jbiotec.2016.07.005).
 - 57 F. Villalobos-Castillejos, V. G. Granillo-Guerrero, D. E. Leyva-Daniel, L. Alamilla-Beltrán, G. F. Gutiérrez-López and A. Monroy-Villagrana, *et al.*, Fabrication of nanoemulsions by microfluidization, in *Nanoemulsions*, 2018, DOI: [10.1016/B978-0-12-811838-2.00008-4](https://doi.org/10.1016/B978-0-12-811838-2.00008-4).
 - 58 A. Naseema, L. Kovooru, A. K. Behera, K. P. P. Kumar and P. Srivastava, A critical review of synthesis procedures, applications and future potential of nanoemulsions, *Adv. Colloid Interface Sci.*, 2021, **287**, 102318, DOI: [10.1016/j.cis.2020.102318](https://doi.org/10.1016/j.cis.2020.102318).
 - 59 S. Mahdi Jafari, Y. He and B. Bhandari, Nano-emulsion production by sonication and microfluidization - A comparison, *Int. J. Food Prop.*, 2006, **9**, 475–485, DOI: [10.1080/10942910600596464](https://doi.org/10.1080/10942910600596464).
 - 60 S. M. Jafari, Y. He and B. Bhandari, Optimization of nano-emulsions production by microfluidization, *Eur. Food Res. Technol.*, 2007, **225**, 733–741, DOI: [10.1007/s00217-006-0476-9](https://doi.org/10.1007/s00217-006-0476-9).
 - 61 L. Bai and D. J. McClements, Development of microfluidization methods for efficient production of concentrated nanoemulsions: Comparison of single- and



- dual-channel microfluidizers, *J. Colloid Interface Sci.*, 2016, **466**, 206–212, DOI: [10.1016/j.jcis.2015.12.039](#).
- 62 Z. Xing, Y. Xu, X. Feng, C. Gao, D. Wu and W. Cheng, *et al.*, Fabrication of cinnamon essential oil nanoemulsions with high antibacterial activities via microfluidization, *Food Chem.*, 2024, **456**, 139969, DOI: [10.1016/j.foodchem.2024.139969](#).
- 63 J. Dimak, N. Somala, C. Laosinwattana and M. Teerarak, The effect of microfluidization on characteristics and herbicidal potential of peppermint nanoemulsion on *Amaranthus tricolor*, *Int. J. Agric. Technol.*, 2024, **20**(1), 77–86.
- 64 J. Wan, S. Zhong, P. Schwarz, B. Chen and J. Rao, Physical properties, antifungal and mycotoxin inhibitory activities of five essential oil nanoemulsions: Impact of oil compositions and processing parameters, *Food Chem.*, 2019, **291**, 199–206, DOI: [10.1016/j.foodchem.2019.04.032](#).
- 65 S. Appah, W. Jia, M. Ou, W. P. Pei and E. A. Asante, Analysis of potential impaction and phytotoxicity of surfactant-plant surface interaction in pesticide application, *Crop Prot.*, 2020, **127**, 104961, DOI: [10.1016/j.cropro.2019.104961](#).
- 66 M. O. Jibrin, Q. Liu, J. B. Jones and S. Zhang, Surfactants in plant disease management: A brief review and case studies, *Plant Pathol.*, 2021, **70**, 495–510, DOI: [10.1111/ppa.13318](#).
- 67 N. Tkachuk, L. Zelena and O. Fedun, Phytotoxicity of the aqueous solutions of some synthetic surfactant-containing dishwashing liquids with and without phosphates, *Environ. Eng. Manag. J.*, 2022, **21**(6), 965–970, DOI: [10.30638/eemj.2022.087](#).
- 68 A. H. Nour, Emulsion types, stability mechanisms and rheology: A review, *Int. J. Innov. Res. Sci. Stud.*, 2018, **1**(1), 11–17, DOI: [10.53894/ijirss.v1i1.4](#).
- 69 M. Liu, Y. Pan, M. Feng, W. Guo, X. Fan, L. Feng and Y. Cao, Garlic essential oil in water nanoemulsion prepared by high-power ultrasound: Properties, stability and its antibacterial mechanism against MRSA isolated from pork, *Ultrason. Sonochem.*, 2022, **90**, 106201, DOI: [10.1016/j.ULTSONCH.2022.106201](#).
- 70 A. Malhotra and J. N. Coupland, The effect of surfactants on the solubility, zeta potential, and viscosity of soy protein isolates, *Food Hydrocolloids*, 2004, **18**(1), 101–108, DOI: [10.1016/S0268-005X\(03\)00047-X](#).
- 71 R. H. Müller, C. Jacobs and O. Kayser, Nanosuspensions as particulate drug formulations in therapy: Rationale for development and what we can expect for the future, *Adv. Drug Delivery Rev.*, 2001, **47**(1), 3–19, DOI: [10.1016/S0169-409X\(00\)00118-6](#).
- 72 M. Heydari, A. Amirjani, M. Bagheri, I. Sharifian and Q. Sabahi, Eco-friendly pesticide based on peppermint oil nanoemulsion: preparation, physicochemical properties, and its aphicidal activity against cotton aphid, *Environ. Sci. Pollut. Res.*, 2020, **27**, 6667–6679, DOI: [10.1007/s11356-019-07332-y](#).
- 73 A. R. Roonjho, R. M. Awang, A. S. Mokhtar and N. Asib, Development of saponin based nano emulsion formulations from *Phaleria macrocarpa* to control *Aphis gossypii*, *J. Adv. Zool.*, 2022, **43**(1), 43–55, DOI: [10.17762/jaz.v43i1.113](#).
- 74 F. Laudani, O. Campolo, R. Caridi, I. Latella, A. Modafferi and V. Palmeri, *et al.*, Aphicidal activity and phytotoxicity of *Citrus sinensis* essential-oil-based nano-insecticide, *Insects*, 2022, **13**, 1150, DOI: [10.3390/insects13121150](#).
- 75 N. E. Papanikolaou, A. Kalaitzaki, F. Karamaouna, A. Michaelakis, V. Papadimitriou and V. Dourtoglou, *et al.*, Nano-formulation enhances insecticidal activity of natural pyrethrins against *Aphis gossypii* (Hemiptera: Aphididae) and retains their harmless effect to non-target predators, *Environ. Sci. Pollut. Res.*, 2018, **25**, 10243–10249, DOI: [10.1007/s11356-017-8596-2](#).
- 76 P. C. Mondal, R. Salim, V. Kumar, P. Kaushik, N. A. Shakil and K. Pankaj, *et al.*, Aphicidal activity of nano-emulsions of spearmint oil and carvone against *Rhopalosiphum maidis* and *Sitobion avenae*, *Sci. Rep.*, 2024, **14**, 24226, DOI: [10.1038/s41598-024-74149-2](#).
- 77 H. M. Metwally, S. S. Ibrahim and E. A. Sammour, Aphicidal and biochemical effects of emulsifiable concentrate and nanoemulsion of two selected essential oils against black bean aphid, *Aphis fabae* (Scop.), *Egypt. Pharm. J.*, 2022, **21**(3), 318–327, DOI: [10.4103/epj.epj_40_22](#).
- 78 N. M. Abdelmaksoud, A. M. El-Bakry, E. A. Sammour and N. F. Abdel-Aziz, Comparative toxicity of essential oils, their emulsifiable concentrates and nanoemulsion formulations against the bean aphid, *Aphis fabae*, *Arch. Phytopathol. Plant Prot.*, 2023, **56**(3), 187–208, DOI: [10.1080/03235408.2023.2178065](#).
- 79 N. M. Abdelmaksoud, A. M. El-Bakry, N. F. Abdel-Aziz, E. A. Sammour and H. A. N. Salem, Potency of emulsifiable concentrate and nanoemulsion formulations as green insecticides against two insects, *Aphis craccivora* and *Liriomyza trifolii*, *Ind. Crops Prod.*, 2024, **208**, 117854, DOI: [10.1016/j.indcrop.2023.117854](#).
- 80 K. Abdelaal, M. Essawy, A. Quraytam, F. Abdallah, H. Mostafa and K. Shoueir, *et al.*, Toxicity of essential oils nanoemulsion against *Aphis craccivora* and their inhibitory activity on insect enzymes, *Processes*, 2021, **9**, 624, DOI: [10.3390/pr9040624](#).
- 81 M. Kah, R. S. Kookana, A. Gogos and T. D. Bucheli, A critical evaluation of nanopesticides and nanofertilizers against their conventional analogues, *Nat. Nanotechnol.*, 2018, **13**(8), 677–684, DOI: [10.1038/s41565-018-0131-1](#).
- 82 Y. Zhang and G. G. Goss, Nanotechnology in agriculture: Comparison of the toxicity between conventional and nano-based agrochemicals on non-target aquatic species, *J. Hazard. Mater.*, 2022, **439**, 129559, DOI: [10.1016/j.jhazmat.2022.129559](#).
- 83 L. A. Hooven, P. Chakrabarti, B. J. Harper, R. R. Sagili and S. L. Harper, Potential risk to pollinators from nanotechnology-based pesticides, *Molecules*, 2019, **24**(24), 4458, DOI: [10.3390/molecules24244458](#).
- 84 E. Mazzara, E. Spinozzi, F. Maggi, R. Petrelli, D. Fiorini and S. Scortichini, *et al.*, Hemp (*Cannabis sativa* cv. Kompolti) essential oil and its nanoemulsion: Prospects for insecticide development and impact on non-target microcrustaceans, *Ind. Crops Prod.*, 2023, **203**, 117161, DOI: [10.1016/j.indcrop.2023.117161](#).



- 85 G. E. Nenaah, A. A. Almadiy, B. A. Al-Assiuty and M. H. Mahnashi, The essential oil of *Schinus terebinthifolius* and its nanoemulsion and isolated monoterpenes: investigation of their activity against *Culex pipiens* with insights into the adverse effects on non-target organisms, *Pest Manage. Sci.*, 2022, **78**(3), 1035–1047, DOI: [10.1002/ps.6715](https://doi.org/10.1002/ps.6715).
- 86 R. S. Esteves, R. Apolinário, F. P. Machado, D. Folly, V. C. R. Viana and A. P. Soares, *et al.*, Insecticidal activity evaluation of *Persea venosa* Nees & Mart. essential oil and its nanoemulsion against the cotton stainer bug *Dysdercus peruvianus* (Hemiptera: Pyrrhocoridae) and pollinator bees, *Ind. Crops Prod.*, 2023, **194**, 116348, DOI: [10.1016/j.indcrop.2023.116348](https://doi.org/10.1016/j.indcrop.2023.116348).
- 87 G. Giunti, G. Benelli, V. Palmeri, F. Laudani, M. Ricupero and R. Ricciardi, *et al.*, Non-target effects of essential oil-based biopesticides for crop protection: Impact on natural enemies, pollinators, and soil invertebrates, *Biol. Control*, 2022, **176**, 105071, DOI: [10.1016/j.biocontrol.2022.105071](https://doi.org/10.1016/j.biocontrol.2022.105071).
- 88 A. Modafferi, A. Urbaneja, C. M. Aure, G. Giunti, I. M. Sanudo, A. Ienco and M. Pérez-Hedo, Metabolic and microbial responses of *Ceratitis capitata* to garlic essential oil-based nanoemulsion: implications for pest management, *Pestic. Biochem. Physiol.*, 2025, 106569, DOI: [10.1016/j.pestbp.2025.106569](https://doi.org/10.1016/j.pestbp.2025.106569).
- 89 P. Calatayud-Vernich, F. Calatayud, E. Simó, M. M. Suarez-Varela and Y. Picó, Influence of pesticide use in fruit orchards during blooming on honeybee mortality in 4 experimental apiaries, *Sci. Total Environ.*, 2016, **541**, 33–41, DOI: [10.1016/j.scitotenv.2015.08.131](https://doi.org/10.1016/j.scitotenv.2015.08.131).
- 90 S. H. Mcart, A. A. Fersch, N. J. Milano, L. L. Truitt and K. Böröczky, High pesticide risk to honey bees despite low focal crop pollen collection during pollination of a mass blooming crop, *Sci. Rep.*, 2017, **7**, 46554, DOI: [10.1038/srep46554](https://doi.org/10.1038/srep46554).
- 91 P. Uhl and C. A. Brühl, The impact of pesticides on flower-visiting insects: A review with regard to european risk assessment, *Environ. Toxicol. Chem.*, 2019, **38**(11), 2355–2370, DOI: [10.1002/etc.4572](https://doi.org/10.1002/etc.4572).
- 92 G. Giuliano, O. Campolo, G. Forte, A. Urbaneja, M. Pérez-Hedo and I. Latella, *et al.*, Insecticidal activity of *Allium sativum* essential oil-based nanoemulsion against *Spodoptera littoralis*, *Insects*, 2024, **15**, 476, DOI: [10.3390/insects15070476](https://doi.org/10.3390/insects15070476).
- 93 M. Ricupero, A. Biondi, F. Cincotta, C. Conduro, V. Palmeri and A. Verzera, *et al.*, Bioactivity and physico-chemistry of garlic essential oil nanoemulsion in tomato, *Entomol. Gen.*, 2022, **42**, 921–930, DOI: [10.1127/entomologia/2022/1553](https://doi.org/10.1127/entomologia/2022/1553).

