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Metabolic and microbial responses of *Ceratitis capitata* to essential oil-based nano-emulsions: Implications for pest management

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ABSTRACT

Essential oils (EOs) are a promising alternative to conventional pesticides, but some challenges like high volatility, poor water solubility, and rapid degradation limit their use in Integrated Pest Management (IPM). To overcome these limitations, this study aimed to develop garlic, eucalyptus, and clove EO-based nano-emulsions (EO-NEs) in a bait treatment format through the high-pressure microfluidization technique and investigated the biological activities against *Ceratitis capitata*. In addition, the adverse effects of the most promising nano-emulsion were evaluated towards a non-target parasitoid *Anagaspis daci*. The garlic nano-emulsion (garlic-NE) with estimated LD_{50} and LD_{90} of 0.96 and 2.18 % of EO showed high insecticidal activity against *C. capitata*. Instead, eucalyptus and clove nano-emulsions exibhited low and mediuam mortality rates respectively. However, garlic-NE exhibited high toxicity (100 % mortality) towards the non-target parasitoid *A. daci*. We analyzed the expression of 14 genes involved in detoxification pathways to investigate the mechanisms underlying garlic-NE toxicity. Our results showed that garlic-NE triggered a strong metabolic detoxification response, leading to the upregulation of cytochrome P450 monooxygenases (*CYPs*), glutathione S-transferases (*GSTs*), and alcohol dehydrogenase (*Adh*). Furthermore, garlic-NE induced significant shifts in the gut microbiota composition of *C. capitata* 72 h after the treatment, disrupting its microbial homeostasis. This dual effect—metabolic stress combined with microbiota disruption—suggests that garlic-NE exerts its insecticidal action through a multi-faceted mode of action rather than direct neurotoxicity, potentially reducing the risk of resistance development. Overall, this study highlights the potential of EO-based nano-formulations as alternatives to conventional pesticides.

1. Introduction

Ceratitis capitata (Wiedemann) (Diptera: Tephritidae), commonly known as the Mediterranean fruit fly (Medfly), is a significant global pest affecting many fruit crops. Its infestations cause substantial economic losses by reducing fruit quality and marketability biology (Blythe et al., 2020; Giunti et al., 2023a; Liquido et al., 1990; Mavrikakis et al., 2000). Despite significant advancements in integrated pest management (IPM) strategies and the adoption of more sustainable methods, such as biological control, the use of bio inputs, and the Sterile Insect Technique (SIT), chemical control remains the primary approach for managing this pest in many parts of the world. However, while effective, the reliance on synthetic pesticides presents several challenges, including the development of resistance, environmental contamination, and adverse effects on non-target organisms, including humans and beneficial insects (Abad-Moyano et al., 2009; Laudani et al., 2022).

Over the last decade, innovative and eco-friendly pest management strategies have been investigated to overcome these challenges for their

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potential application against *C. capitata* (Giunti et al., 2023b; Ortolá et al., 2024). Using natural antagonists such as predators and parasitoids represents a promising approach. Indeed, different authors reported the prey activity of different species of ants, carabids, wasps, spiders, and others (Garcia et al., 2020; Hendrichs et al., 1994; Urbaneja et al., 2006). In addition, more than 60 parasitoids belonging to Braconidae, Chalcididae, Diapriidae, Eulophidae, Figitidae, and Pteromalidae families were reported as Medfly parasitoids (Giunti et al., 2023b). Despite the large number of predators and parasitoids, biological control alone cannot reduce the huge damage to fruits caused by this pest (Giunti et al., 2023b).

Among the green control strategies to manage *C. capitata*, the use of botanicals, in particular EOs, as insecticides is considered an innovative strategy (Campolo, 2022). In this context, several authors reported the efficacy of these substances against the Medfly. Benelli et al. (2012) studied the ingestion, topical, and fumigation activity of different EOs, highlighting a good insecticidal activity, mainly through topical application, with a mortality rate of around 70 % at 0.1 μL/fly for *Rosmarinus officinalis* L., *Lavandula angustifolia* Millerand, and *Thuja occidentalis* L.. Similarly, Miguel et al. (2010) investigated the biological activities of three EOs (*Mentha pulegium* L., *Thymbra capitata* (L.) Cav., and *Thymus albicans* Hoffmanns. & Link) against *C. capitata* adults, showing a high insecticidal activity of *M. pulegium* EO with a mortality rate of 90 %. The toxicity of EOs was also proven against *C. capitata* larvae. For instance, through ingestion toxicity trials, Papachristos et al. (2009) highlighted good insecticidal activity for different citrus EOs.

The efficacy of the EOs can be attributed to their ability to interfere with some physiological traits of insects (i.e., nervous system, antioxidant defence enzymatic systems, dysregulation of insect development, and cuticular permeability), depending on EO composition and concentration (Popescu et al., 2024; Priestley et al., 2003; Zhou et al., 2008). For instance, EOs are known to affect the nervous systems of insects (Jankowska et al., 2018). In this context, insects treated with EOs inhibited the acetylcholinesterase (*AchE*) activity (Abdelgaleil et al., 2009; Seo et al., 2014; Yeom et al., 2015). In addition, the inhibitory activities of EOs on octopamine and GABA receptors and transient receptor potential (TRP) channels were also confirmed (Blenau et al., 2012; Mossa, 2016; Shaaya and Rafaeli, 2007). Park et al. (2016) demonstrated that certain EO compounds, such as perilla aldehyde and thymol, can inhibit AChE activity by approximately 60 %. Another important aspect related to the efficacy of the EOs as insecticides is their presumed ability to affect the gut microbiome of the insects (Dehghankar et al., 2021; Szczepanik et al., 2018). Akami et al. (2019b), for example, highlighted that *Lippia adoensis* Hochst. EO affects the richness, diversity, and abundance of Proteobacteria, Firmicutes, and Bacteroidetes on *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae). The insect gut microbiome plays a crucial role in digestion, immune system function, and detoxification of xenobiotics, influencing insecticide resistance (Douglas, 2015; Engel and Moran, 2013). Disrupting these microbial communities could compromise the insect's ability to metabolize toxic compounds, affecting digestion, nutrient absorption, and overall resilience to chemical stressors. Therefore, investigating the interplay between metabolic detoxification pathways and microbiome alterations is essential to understanding the mode of action of these natural substances. Despite the promising results of the EOs as insecticides, their practical application is a challenge due to some negative characteristics of EOs, such as volatility, low water solubility, rapid degradation under environmental conditions, and flammability (Campolo, 2022; Modafferi et al., 2024c). However, in the last decade, the use of nanotechnology to overcome these challenges increased the interest of the different stakeholders (Campolo, 2022; Koul, 2019). Nanotechnology allows nano-formulations characterized by unique chemical, physical, and biological attributes. These include: (i) enhanced water solubility, (ii) improved surface coverage, (iii) gradual release of active ingredients (a.i.), (iv) reduced degradation of a.i., (v) minimized phytotoxic effects, (vi) increased permeability through insect cuticles, and (vii) boosted bioactivity (Campolo, 2022; Pavoni et al., 2020). Botanical-based nano-formulations used for insecticidal applications can be achieved through two main approaches, namely bottom-up (e.g., self-emulsification process or phase inversion temperature) and top-down (e.g., sonication, high-pressure homogenization or microfluidization) (Donsì and Ferrari, 2016; Nirmala et al., 2022). Despite several authors reporting the efficacy of both approaches, top-down methods are generally preferred due to some advantages such as industrial scalability, low amount of co-formulants used in the formulations, and optimal physical characteristics (i.e., size, PDI, and surface charge) and stability of developed nano-formulation (Campolo et al., 2020a; Modafferi et al., 2024b).

In this context, this study aimed to develop three different essential oil-based nano-emulsions (EO-NEs) (i.e., garlic-NE, eucalyptus-NE, and clove-NE) through a top-down approach (microfluidization) using a high EO: surfactant ratio (3: 1) and to investigate the biological activity against *C. capitata* adults. The physical properties (particle size, PDI, and surface charge) of EO-NEs were assessed through dynamic light scattering (DLS) analysis. The efficacy of the developed EO-NEs was evaluated against the target insect through ingestion trials when applied in bait formulations to simulate practical field use conditions and assess their potential applicability. At the same time, the adverse effects of the most effective EO-NE (i.e., garlic-NE) against the target pest were evaluated towards one of the most promising fruit-fly biological control agents: the larval-pupal parasitoid *Anagaspis daci* (Weld) (Hymenoptera: Figitidae) (de Pedro et al., 2016). Additionally, the expression of genes involved in different detoxification mechanisms and the effect on the gut microbiome of *C. capitata* were evaluated.

2. Materials and methods

2.1. Insect rearing

Ceratitis capitata and *A. daci* were reared for several generations in the laboratories of the IVIA. Specifically, the Medfly was reared according to the methods described in (Jacas Miret et al., 2008).The colony was maintained under constant climatic conditions (25 ± 2 °C, 60–70 % RH with a photoperiod of 14 h:10 h L:D) and was reinforced by regular additions of flies emerging from pupae collected from naturally infested fruits in the field. Adults were fed with a mixture of sugar and hydrolyzed yeast extract (ratio 4:1 w:w) and water ad libitum.

Aganaspis daci was reared according to the methods described by de Pedro et al. (2018), and the original colony was obtained from Medfly larvae collected from figs in a nearby Valencian village (Bétera, Spain). Wasp adults were reared in plastic cages (35 × 30 × 35 cm) provided with water, sugar, and honey as food sources under constant climatic conditions (22 ± 2 °C, 65 ± 5 % RH, and 16 h:8 h L:D photoperiod). *C. capitata* pupae were used as host species. For both species, newly emerged adults (0–2 days) were used in the experiments.

2.2. Chemical characterization of EOs

The *Allium sativum* L. EO (garlic) and *Syzygium aromaticum* (L.) Merr. & L.M.Perry EO (clove) were purchased from Esperis S.p.A. (Milan, Italy). *Eucalyptus camaldulensis* (Dehnh) EO (eucalyptus) was steam-extracted from leaves collected from plants growing in southern Italy (Reggio Calabria, Italy). The chemical characterization of the EOs was carried out following the methods described by Giunti et al. (2019). 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 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 were injected at a split ratio of 1:30. The identification of peaks was

made using computer matching against the commercial libraries (NIST 05, Wiley FdaviesFNSC and ADAMS) comparing linear retention indices (LRI). The LRIs were calculated using the formula of Van Den Dool and Kratz, 1963 by comparing the retention times of the compounds to be identified with those of a standard mixture of alkanes (C8-C20 saturated alkanes standard mixture, Supelco®, Bellefonte, PA, USA) which was analyzed in GC–MS under the identical operating conditions as the sample (Van Den Dool and Kratz, 1963; Masada, 1976; McLafferty et al., 1989; Davies, 1990; Jennings, 2012; Adams, 2017).

2.3. EO-NEs: Formulation and characterization

All the EO-NEs (i.e., garlic, eucalyptus, and clove-NE) were formulated and characterized in the laboratories of *Entomologia Generale e Applicata*, Department of *AGRARIA*, University of Reggio Calabria, Italy.

The EO-NEs were obtained according to the methods described by Modafferi et al. (2024c). First, the EO and tween 80® (polyoxyethylene (20) sorbitan monooleate, Sigma Aldrich, Munich, Germany) (ratio 3:1) were mixed for 5 min at 7000 RPM through a magnetic stirrer (MRS scientific Ltd., U.K.). Then, double-stilled water was added slowly (1 mL min^{-1}) to the previous solution (EO and surfactant). The obtained emulsion (EO 15 % w/w; tween 80® 5 % w/w; water 80 % *w*/w) was homogenized five times using a high-pressure microfluidizer device (HPM) (LM20 Microfluidizer™ Processor, USA) at 30.000 PSI. The resulting EO-NEs were collected in aluminum bottles and stored at 4 °C. The EO-NEs were physically characterized through Dynamic Light Scattering (DLS) analysis to assess particle size (nm), the polydispersity index (PDI), and surface charge (ζ-potential). The analyses were performed by diluting the obtained EO-NEs in double-distilled water (ratio 1:200) 24 h after the development.

2.4. Insecticidal activity of developed EO-NEs

2.4.1. Ceratitis capitata

The insecticidal activity of the developed EO-NEs was evaluated through ingestion trials against *C. capitata* adults (Juan-Blasco et al., 2013). In detail, the trials were carried out inside plastic boxes (5 × 10 × 10 cm) in which ten newly emerged Medflies (sex ratio 1:1) were fed daily with four drops (50 μL each) of the tested formulations until the end of the experiment (72 h). To evaluate the efficacy of each EO-NE (i.e., garlic, eucalyptus, and clove-NE) against the target insect and to select the most effective one, an initial experiment was conducted in which the insects were offered a dilution of the developed EO-NE containing a high concentration of EO (i.e., 2.5 %) as active ingredient (a.i.). The desired dilution was prepared by diluting the required amount of EO-NE in a sucrose-water solution (30 % w/w) to simulate the practical conditions under which insecticides are used in bait formulations for pest management programs against *C. capitata* (Chueca et al., 2007). Bioassays were performed under the same rearing conditions (25 ± 2 °C, 60–70 % RH with a photoperiod of 14 h:10 h L:D). Insects were considered dead if they were unable to fly. Eight replicates were performed for each dilution; sucrose water solution (30 % w/w) and a bait insecticide (Spintor™ Fly) at label concentration (0.048 g/L) were used as a negative control (C-) and positive control (C+), respectively. This experiment demonstrated that garlic-NE was the most effective formulation, and therefore, it was the only one subjected to a serial dilution bioassay (1.87, 1.25, 0.93, 0.625, and 0.46 % w/w of EO). The other nano-formulations were discharged, as the concentrations required to control Medfly adults were not viable for practical application.

2.4.2. Aganaspis daci

The toxic effects of selected garlic-NE towards *A. daci* adults were evaluated through the above-mentioned ingestion trials, using the estimated LD_{50} (0.96 % of EO) and LD_{90} (2.16 % of EO) against *C. capitata* adults (see Table 2), sucrose water solution (30 % w/w) as negative control (C-) and Spintor™ Fly at label concentration (0.048 g/L) as positive control (C+). Preliminary trials highlighted no mortality between the sucrose-water and sucrose-water-tween 80 solutions. The trials, replicated ten times, were carried out inside plastic Petri dishes (9 cm Ø) with a hole in the lid (3 cm Ø) sealed with muslin. Five *A. daci* adults were carefully placed inside the arena and fed daily with four drops (50 μL each) of the tested formulation or controls. Arenas were maintained inside a climate chamber at 22 ± 2 °C, 65 ± 5 % relative humidity (RH), and 16 h: 8 h (light: dark L: D) photoperiod. Mortality was recorded 72 h after treatments. Insects were considered dead if they could not fly or remained immobile when stimulated with a fine brush.

2.5. Effects on detoxification genes of C. capitata

To evaluate the effects of garlic-NE on insect detoxification pathways, the transcriptional levels of 13 genes involved in different metabolic processes were analyzed via real-time PCR (qPCR) (Table 1). Three treatments were applied using the estimated LD_{50} (0.96 % EO) and LD_{90} (2.18 % EO) against *C. capitata* (see Table 2), along with a negative control (C-). The analyzed genes were categorized based on their roles in detoxification pathways and neurotransmitter metabolism.

- Phase I detoxification (Modification of xenobiotics): This phase involves genes encoding Cytochrome P450 monooxygenases (CYPs) (*Cyp6g2, Cyp6a2, Cyp6a69, Cyp12e1*), which catalyze oxidation reactions to enhance the solubility of xenobiotics, facilitating their subsequent metabolism and excretion. Alcohol Dehydrogenase (Adh) was included, as it plays a crucial role in the oxidation of alcohol-derived compounds, contributing to xenobiotic detoxification.
- Phase II detoxification (Conjugation and solubilization): This phase involves genes encoding Glutathione S-transferases (GSTs) (*GSTS1, MGSTL1*), which conjugate toxic compounds with glutathione,

Table 1
Primer sequences for target and housekeeping genes used in the transcriptional analysis of *Ceratitis capitata* detoxification pathways.

Gene	Gene description	Primer sequence (5′-3′)
TUB1a (HK)	Tubulin Alpha	FW: ACATTACTATCCGGCTACACAG RV: TGATAATTCTTCGAGCGTCCAC
AANAT	Aralkylamine N-Acetyltransferase	FW: GTCGGATTCATGCCAACACC RV: GCTATGCCCAGTCCACGAT
AChE	Acetylcholinesterase	FW: TTTGCAGGCACCCTCGTC RV: ACCACGACCGATGTCAC
Adh	Alcohol Dehydrogenase	FW: AATGAATCGGCTTACAACGAAA RV: TTCCTCACATATGCCAACACC
GABAT	Gamma-Aminobutyric Acid Transaminase	FW: AAGAACTTTAGTCAACCGTCCT RV: TTCGAACACGATCCGCACA
GSTS1	Glutathione S Transferase S1	FW: TTCGATGGCCCGCTTT RV: ATCTCATCCTCCGGTTCGT
MGSTL1	Microsomal Glutathione S-Transferase 1	FW: GGATTTATTGGACAAACGCCTGAA RV: CACATAAAGGAAACCAATGACGAAG
TBH	Tyramine Beta Hydroxylase	FW: CACCACAGCCATTGCCGAG RV: AGCCAAATCTTCACGAGCTT
Cyp6g1	Cytochrome P450 6 g1	FW: CGAACACCTTTGACCCGGAA RV: GCACAAGTCGCAACATAGTGA
Cyp6g2	Cytochrome P450 6 g2	FW: CGCCAGCCTCTTCCGA RV: CGTAAATGACGCCACTAGCA
Cyp6a2	Cytochrome P450 6a2	FW: ACCGGAACGCCTCGAC RV: AGCGCAGACCTATGCAA
Cyp6a69	Cytochrome P450 6a69	FW: TTGCGCCTTCGGACTCGAG RV: TAGAAATTGTGCAGCGGTTGTGGT
Cyp6t3	Cytochrome P450 6 t3	FW: ATCGCGTTGATTTTATACGAGCTG RV: GCCTTCCGCAACCACC
Cyp12e1	Cytochrome P450 12e1	FW: CCGGCACAGACATATCCATC RV: TCATTACGCAGCCAACGTTC

Table 2
Physical characteristics (size, PDI, and zeta potential) of developed EO-NEs 24 h after development. Values are the mean (± standard deviation) of three replicates. Different letters indicate statistical differences among the EO-NEs within the same physical properties ($P < 0.05$; ANOVA).

EO-NE[1]	Size (nm)	PDI[2]	Zeta Potential (mV)
Garlic-NE	61.7 ± 0.28 a	0.049 ± 0.003 a	−11.1 ± 0.21 a
Eucalyptus-NE	63.86 ± 0.34 a	0.088 ± 0.013 b	−11.0 ± 0.46 a
Clove-NE	179.9 ± 2.21 b	0.175 ± 0.016 c	−23.4 ± 0.05 b
(*F*; df; *P level*)	(8065.05; 2; <0.001)	(87.31; 2; <0.001)	(103.19; 2; <0.001)

[1] Essential oil-based nano-emulsion.
[2] Polydispersity index.

enhancing their solubility and promoting their elimination from the organism.

- Neurotransmitter metabolism and synaptic regulation: Several neurotransmission-related genes were also analyzed. Acetylcholinesterase (AChE) degrades acetylcholine at the synapse, regulating neural signaling. Tyramine beta-hydroxylase (TBH) catalyzes the conversion of tyramine into octopamine, a key neurotransmitter involved in insect neuromodulation. Lastly, Gamma-aminobutyric acid transaminase (GABAT) plays a role in the degradation of gamma-aminobutyric acid (GABA), the primary inhibitory neurotransmitter in insects, which is crucial for maintaining neural homeostasis.

The TUB1a (*tubulin1-alpha*) gene was used as a housekeeping gene for normalization (Ponton et al., 2011). Each treatment was replicated ten times, following the protocol described in section 2.4.1.

Twenty-four hours after the treatment, the Medflies were placed inside an Eppendorf (2 mL), immediately ground in liquid nitrogen, and then stored at −80 °C until the RNA extraction. Following the manufacturer's recommendations, total RNA was extracted using NZYol (NZYTech, Lisboa, Portugal). Then, 10 μg of total RNA was treated with a TURBOTM DNA-free kit (Ambion, Life Technologies, CA, United States) to remove the DNA contamination. Reverse transcription was performed using PrimeScript RT reagent kit (TAKARA BIO INC., Japan) starting from 1 μg of RNA-DNA free template. Real-time PCR amplification was performed in LightCycler 480 System (Roche Molecular Systems, Inc., Switzerland), using 5 μl of NZYSupreme qPCR Green Master Mix (2×) (NZYTech, Lisboa, Portugal), 0.5 μL of forward and reverse primers (10 mM) and 2 μL of cDNA, resulting in a total reaction volume of 10 μL. The reaction was performed using the following PCR conditions: 1 cycle at 95 °C for 2 min, 40 cycles at 95 °C for 15 s, 58 °C for 30 s, 72 °C for 30 s, and a final melting cycle at 95 °C for 5 s and 65 °C for 1 min. The relative fold gene expression of the samples was calculated using the $2^{\Delta\Delta CT}$ method described by Ricupero et al. (2023).

2.6. Effects of garlic-NE on C. capitata microbiome

The influence of the garlic-NE treatment on the bacterial communities of *C. capitata* adults was evaluated using the above-mentioned ingestion trials (see section 2.4.1). A total of three treatments were carried out using the estimated LD_{30} (0.69 % of EO) (see Table 3), sucrose water solution (30 % w/v) (C-), and a sucrose/water/tween solution (30/65/5 % w/*v*/w) (Ct). Four replicates were performed for each treatment with a pool of three medflies each. In detail, for every treatment, the specimens were fed daily with four drops (50 μL each) until the end of the experiment (72 h). After 24 and 72 h, the insects were placed into Eppendorf tubes (1.5 mL), immediately ground in liquid nitrogen, and stored at −80 °C until use. Firstly, the samples were immersed in sodium hypochlorite solution (0.1 %) and then in sterile water to sterilize the insect's surfaces. Total DNA was extracted using the NZY Tissue Gdna isolation kit (Nzytech) kit following the manufacturer's protocol. Bacterial communities were characterized through the amplification of the hypervariable region V3 and V4 using primers 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATC). Amplicon library preparation was performed according to recommended protocols (Illumina Demonstrated Protocol: 16S Metagenomic Sequencing Library Preparation) and then sequenced on an Illumina Miseq by Macrogen (Netherlands).

Table 3
Estimated lethal doses (LD_{30}, $LD_{50,}$ and LD_{90}) of garlic-NE against *Ceratitis capitata* adults. Values were considered statistically different if their 95 % fiducial limits did not overlap.

LD[1]	Estimate	95 % Confidence Limits garlic-NE doses %		*X* (df[2])	*P* level
		Lower bound	Upper bound		
LD_{30}	0.69	0.61	0.76		
LD_{50}	0.96	0.87	1.06	7.438 (3)	0.19
LD_{90}	2.18	1.91	2.55		

[1] Lethal dose.
[2] Degrees of freedom.

2.7. Data analysis

Statistical analyses were performed using IBM® SPSS® Statistics v. 23 (IBM Corp. Released 2015. Armonk, NY, USA). All data met the assumptions required by parametric tests, including normality and homoscedasticity of variance ($P > 0.05$). Differences among the developed EO-NEs (i.e., garlic, eucalyptus, and clove-NE) in the physical characteristics were subjected to analysis of variance (ANOVA) with size, PDI, and surface charge as dependent variables, and EO-NEs used as fixed factors. *Ceratitis capitata* mortality data were corrected for control mortality using Abbott's formula (Abbott, 1925). The ANOVA test was used to assess the difference among the treatments in the preliminary test, the serial dilutions trial against *C. capitata,* and the toxicity bioassay towards a natural antagonist such as *A. daci*. The data obtained in the serial dilution bioassay were subjected to a probit analysis to estimate the LD_{50} and LD_{90} values and their 95 % fiducial limits. The statistical differences among the treatments (i.e., C-, $LD_{50,}$ and LD_{90}) on the transcription level of *C. capitata* genes were subjected to ANOVA analysis.

The raw 16S rRNA gene amplicon data representing the Medfly bacterial communities were analyzed in RStudio v4.2.2 using the DADA2 R package (Callahan et al., 2016). Read-quality profiles were inspected, and low-quality reads were subsequently filtered and deleted. The selected sequences were merged, dereplicated, and classified into unique Amplicon Sequence Variants (ASVs). ASVs with 100 % sequence identity were preferred over operational taxonomic units (OTU) due to their superior reproducibility and resolution (Callahan et al., 2017). The ASVs identified as mitochondria or chloroplasts were removed from the dataset. Taxonomic classification of the ASVs was performed using a classify-by-consensus approach, with Silva SSU (v. 138.1) as the reference database, (Quast et al., 2013) and normalized using the Cumulative Sum Scaling (CSS) method. The MicrobiotaProcess package was used to perform alpha- and beta-diversity analyses and to identify the key taxa comprising the core microbiome (Xu et al., 2023). Alpha diversity, which assesses within-sample diversity, was evaluated using the Chao1, Shannon, and Simpson 1-D indices. Beta diversity was evaluated using analysis of similarities (ANOSIM) (Anderson and Walsh, 2013), and permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001), using 999 permutations with the "adonis" function. The dispersion among the groups was evaluated by multivariate homogeneity of group dispersion test (PERMDISP) (Anderson, 2006), using the "betadisper" function. All analyses were carried out using the vegan package (Oksanen et al., 2015). To underline shared ASVs among the groups, Venn diagrams were made using ggplot2 R-package (Wickham, 2009). The sequencing data are available in the NCBI database under

project number PRJNA1214852.

3. Results

3.1. Chemical characterization of EOs

A total of 23, 26, and 63 compounds corresponding to 97.87 %, 99.45 %, and 95.33 % of the total area, were detected in garlic, clove, and eucalyptus EOs, respectively. Garlic EO was composed only of sulfur compounds, clove EO was mainly comprised of oxygenated monoterpenes (88.65 %), and eucalyptus EO was mainly composed of monoterpenes (61.03 %). See Modafferi et al. (2025) for complete analytical procedures and chemical characterizations.

3.2. Physical characterization of developed EO-NEs

The size and zeta potential distribution of the developed EO-NEs, such as garlic-NE, eucalyptus-NE, and clove-NE, are shown in Supp. Figs. 1,2, and 3, respectively. The analysis of their physical characteristics confirmed that all EO-NEs exhibited particle size within the nanoscale range (< 200 nm), with low polydispersity index (PDI) values (<0.2) and negative surface charge, indicating good stability due to repulsion among particles. Notably, garlic-NE and eucalyptus-NE had the smallest particle sizes and the lowest PDI values, while clove-NE showed comparatively larger particle sizes and higher PDI. Regarding surface charge, clove-NE exhibiessted the lowest zeta potential among the tested nano-emulsions. Statistical differences were observed among all EO-NEs for the analyzed physical characteristics ($P < 0.05$) (Table 2).

3.3. Insecticidal activity of developed EO-NEs

The initial bioassay using a high concentration of active ingredient (2.5 % EO) revealed significant differences in the toxicity of the developed EO-NEs against *C. capitata* adults. Garlic-NE exhibited the highest efficacy, reaching nearly 100 % mortality at 72 h post-exposure. In contrast, clove-NE induced an intermediate mortality rate, while eucalyptus-NE showed the lowest toxicity throughout the experiment. Statistical analysis confirmed significant differences among treatments 48 and 72 h after the exposure ($F = 21.95$; df = 2; $P < 0.001$ and $F = 38.02$; df = 2; $P < 0.001$, respectively), with garlic-NE consistently outperforming the other formulations (Fig. 1).

The dose-response bioassay confirmed a significant positive correlation between garlic-NE concentration and *C. capitata* adult mortality at 72 h post-exposure (Fig. 2). The experimental data highlighted a dose-dependent mortality, with statistical differences observed among the tested doses ($F = 26.1$; df = 1, 4; $P < 0.01$). Mortality increased progressively with higher EO concentrations, reaching nearly 90 % at the highest tested dose (1.87 % EO). Mortality rates at 0.46 % and 0.625 % EO remained low and statistically similar, whereas doses of 0.93 % EO and above significantly increased insect mortality. Furthermore, the experimental data fitted with the Probit model ($X^2 = 7.438$; df = 1, 3; $P = 0.19$), and the lethal doses and their fiducial limits were estimated (Table 3).

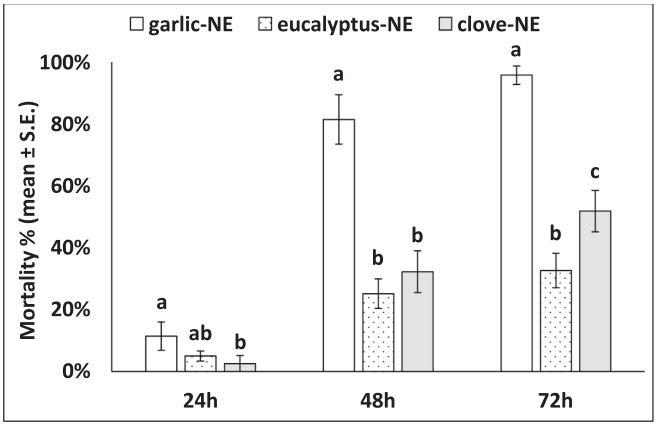

Fig. 1. Mortality % (mean ± SE) of *C. capitata* adults at 24, 48 and 72 h after exposure to garlic, eucalyptus, and clove NEs (2.5 % of EO). Different letters indicate significant differences among treatments within the same exposure time ($P < 0.05$).

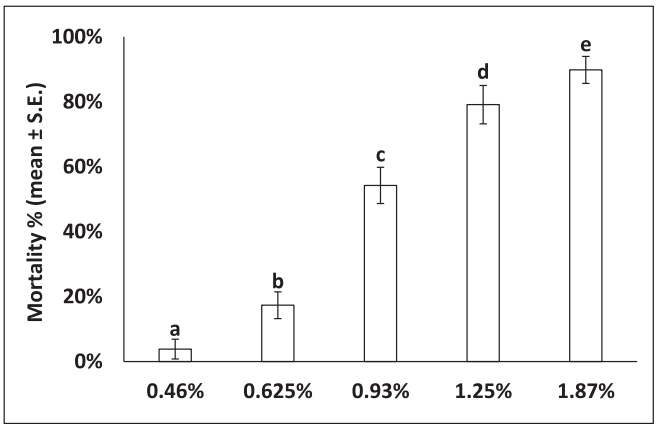

Fig. 2. Dose-response mortality (mean ± SE) of garlic-NE dilutions (% of EO) against *Ceratitis capitata* adults 72 h after exposure. Different letters indicate statistical differences among the doses ($P < 0.05$).

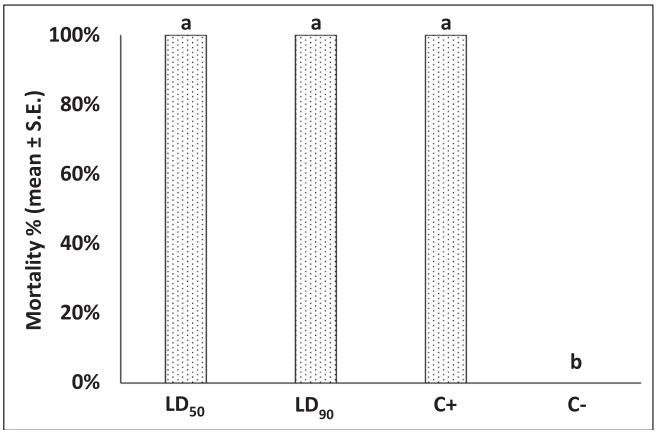

Fig. 3. Mortality % (mean ± SE) of *Aganaspis daci* adults 72 h after exposure to garlic-NE at LD_{50}, LD_{90}, positive control (C+; Spintor™ Fly), and negative control (C-; sucrose water solution 30 % w/v). Different letters indicate significant differences among treatments ($P < 0.05$).

The toxicity of garlic-NE against *A. daci* adults at 72 h post-exposure is presented in Fig. 3. The results demonstrated a highly lethal effect, with 100 % mortality observed for both tested doses (LD_{50} = 0.87 % EO and LD_{90} = 1.91 % EO). No significant differences were detected between the lethal doses and the positive control (C+; Spintor™ Fly). No mortality was recorded in the negative control (C-; sucrose water solution 30 % w/v).

3.4. Impact of garlic-NE on gene expression against C. capitata

The study of transcriptional levels of the genes *AANAT*, *AChE*, *AdH*, *GABAT*, *GSTS1*, *MGSTL1*, *TBH*, *Cyp6g1*, *Cyp6g2*, *Cyp6a2*, *Cyp6a69*, *Cyp6t3*, and *Cyp12e1* highlighted statistical differences among the different treatments (i.e., C-, LD_{50}, and LD_{90}), particularly in the genes *Adh*, *GSTS1*, *Cyp6g2*, *Cyp6a2*, and *Cyp6a69* ($P < 0.05$) (Table 4). The *Adh* gene, involved in Phase I detoxification and alcohol metabolism,

Table 4
Relative expression HK/gene (mean ± standard error) of different *Ceratitis capitata* genes 24 h after the treatment with C- (sucrose-water solution 30 % w/v), LD_{50} (0.96 % of EO), and LD_{90} (2.18 % of EO) of garlic-NE. Transcriptional levels were normalized to the expression of housekeeping (*TUB1a*). Different letters indicate statistical differences among the treatments within the same gene ($P < 0.05$).

Gene	Treatment	Relative expression (± S.E.)	F (df^1=2)	P
Adh	C-	0.66 ± 0.05 a	38.06	<0.001
	LD_{50}	1.15 ± 0.04 b		
	LD_{90}	1.32 ± 0.09 c		
GSTS1	C-	1.13 ± 0.07 a	6.62	0.005
	LD_{50}	1.57 ± 0.11 ab		
	LD_{90}	1.61 ± 0.08 b		
Cyp6g2	C-	1.12 ± 0.16 a	5.31	0.01
	LD_{50}	1.90 ± 0.22 b		
	LD_{90}	1.21 ± 0.16 a		
Cyp6a2	C-	0.85 ± 0.12 a	7.62	0.002
	LD_{50}	1.47 ± 0.15 b		
	LD_{90}	1.49 ± 0.12 b		
Cyp6a69	C-	0.83 ± 0.27 a	3.36	0.014
	LD_{50}	1.77 ± 0.21 b		
	LD_{90}	1.95 ± 0.37 b		

[1] Degrees of freedom.

exhibited a dose-dependent response, with significant differences among treatments. The glutathione S-transferase gene (*GSTS1*) showed a significant overexpression only at the highest concentration (LD_{90}), indicating an activation of Phase II detoxification. In contrast, the Cytochrome *P450* gene (*Cyp6g2*), involved in the oxidative metabolism of xenobiotics, was significantly upregulated at the LD_{50} dose. At the same time, *Cyp6a2* and *Cyp6a69*, also part of the Phase I detoxification, were overexpressed at both tested doses compared to the control. Notably, the increase in *Cyp6a69* expression at LD_{50} and LD_{90} was nearly twice that of the control. The expression of the genes that did not highlight statistical differences among the different treatments is summarized in Supp. Table 1.

3.5. Effects of garlic-NE on C. capitata microbiome

The bacterial community composition of *C. capitata* was analyzed at 24 and 72 h post-treatment. Across all conditions, *Enterobacteriaceae*, particularly the genus *Pluralibacter*, dominated the microbiota. When *Pluralibacter* spp. was excluded, treatment-related differences became more apparent (Fig. 4).

At 24 h, all groups (C-, LD_{30}, Ct) were predominantly composed of *Erwiniaceae*, but secondary bacterial families varied. LD_{30} showed a higher relative abundance of *Sphingomonadaceae* and *Halomonadaceae*, C- exhibited *Enterococcaceae* and *Pseudomonadaceae*, while Ct was enriched in *Rhizobiaceae* and *Halomonadaceae*. By 72 h, *Erwiniaceae* remained dominant, but microbial composition changed: LD_{30} showed an increased proportion of *Staphylococcaceae* and *Moraxellaceae*, C- had a higher presence of *Enterococcaceae*, and Ct displayed enrichment in *Halomonadaceae* and *Burkholderiaceae* (Fig. 4).

Despite these compositional variations, alpha diversity indices (Chao1, Shannon, and Simpson) did not show significant differences among treatments at 24 h ($P > 0.05$, Fig. 5). At 72 h, only the Chao 1 index showed a significant value ($P > 0.05$). Similarly, beta diversity analysis revealed no significant differences at 24 h. At 72 h, the microbial community structure differed among treatments, as indicated by PERMANOVA ($P = 0.05$) and ANOSIM ($P = 0.016$). PCA and PCoA visualizations further supported this finding, showing distinct clustering patterns at 72 h. To assess bacterial community differences within each treatment between 24 and 72 h, alpha and beta diversity were calculated, revealing no significant differences.

Temporal analysis within each treatment showed a decline in shared amplicon sequence variants (ASVs) over time. At 24 h, 19 % of ASVs ($n = 16$) were shared across treatments, with C- (20 %), Ct (32 %), and LD_{30} (18 %) having similar proportions of unique ASVs. At 72 h, the proportion of shared ASVs remained stable (19 %), but their absolute number was reduced by half ($n = 8$). Notably, LD_{30} retained a higher proportion of unique ASVs (37 %) compared to C- (16 %) and Ct (9.3 %) (Fig. 6). Beta diversity analysis across the entire dataset, considering both time and treatment variables, showed no significant differences. However, when *Pluralibacter* spp. was excluded, a significant interaction between treatment and time was detected (PERMANOVA $P < 0.05$; ANOSIM $P < 0.05$). This suggests garlic-NE induces gradual shifts in the *C. capitata* microbiome, with more pronounced effects at 72 h post-treatment.

4. Discussion

To our knowledge, this is the first study demonstrating the toxic effects of EO-NEs against *C. capitata* adults. The biological activity of these formulations depends on several factors, including plant species, EO concentration, application rate, and physical properties such as particle size, PDI, and surface charge. Among these, particle size plays a key role. As noted by Modafferi et al. (2024b), only nano-formulations with

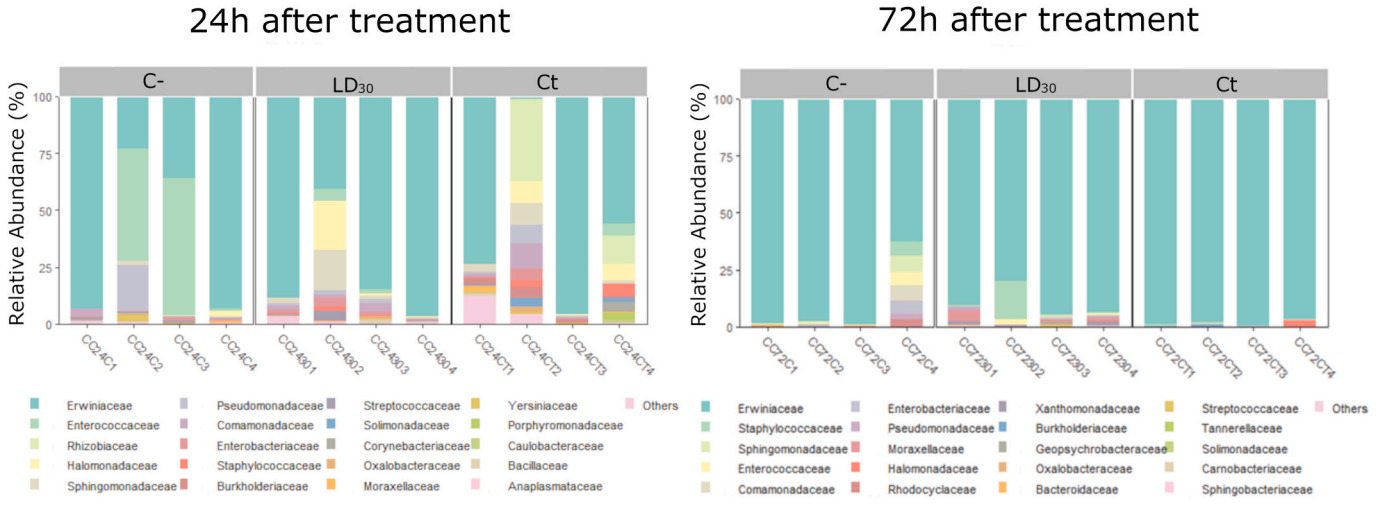

Fig. 4. Bacterial community composition of *Ceratitis capitata* after garlic-NE treatment: Relative abundance of bacterial families at 24 and 72 h post-treatment, after excluding the taxon *Pluralibacter* spp.. Treatments: C- (sucrose/water solution 30 % w/v), LD_{30} (0.69 % EO), and Ct (sucrose/water/Tween 80 solution 30/65/5 % w/v/w).

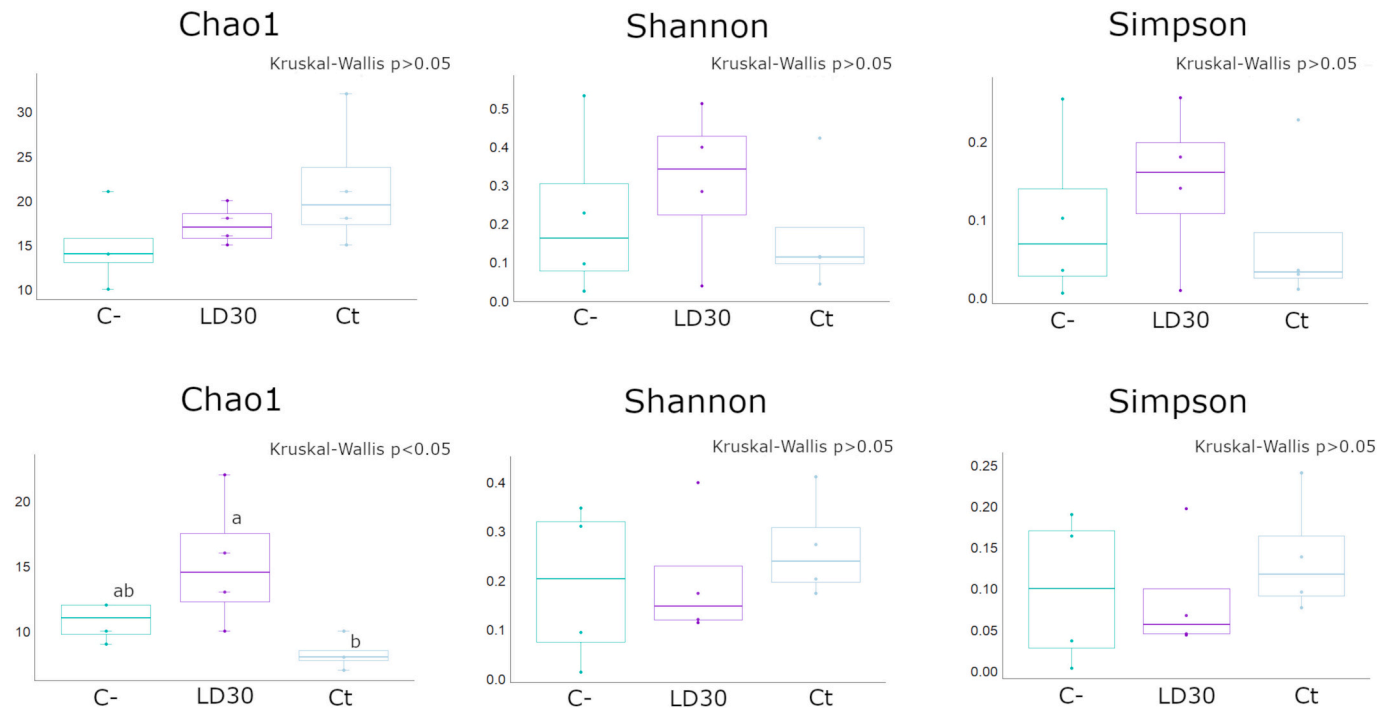

Fig. 5. Different alpha diversity indexes (i.e., Chao1, Shannon, and Simpson) after 24 h (A) and 72 h (B), in *Ceratitis capitata* treated and untreated samples (i.e., C-, LD_{30}, and Ct).

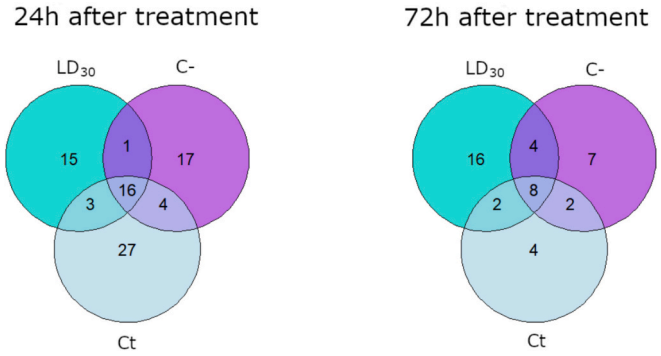

Fig. 6. Venn diagrams showing the number of shared and unique taxa among *Ceratitis capitata* treated and untreated samples (i.e., LD_{30}, C-, and Ct) after 24 h and 72 h, at the genus taxonomic level.

particle sizes <250 nm significantly impact insect survival, unlike larger-sized formulations. Several studies linked nanoparticle size to insecticidal efficacy. Giuliano et al. (2024) developed a garlic EO-based nanoemulsion with larger particles (141.0 ± 1.375 nm) than in our study (61.7 ± 0.28 nm), yet reported high insecticidal activity against *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) (LD_{50}: 1.57 % and LD_{90}: 2.39 % EO). Similarly, Hassan et al. (2023) formulated a clove NE (158.4 ± 0.353 nm), close in size to ours (179.9 ± 2.21 nm), which showed good activity against adult fleas (LD_{50}: 26.42 and LD_{90}: 49.16 µg/ml). Other authors developed eucalyptus EO-based NEs with much smaller droplets (<10 nm) than our eucalyptus-NE (63.86 ± 0.34 nm) and reported good insecticidal activity against *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), *Culex quinquefasciatus* Say (Diptera: Culicidae), *Sitophilus oryzae* (Linnaeus, 1764) (Coleoptera: Curculionidae), *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrichidae), and *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) (Mohammed and Nasr, 2020; Moustafa et al., 2015; Sugumar et al., 2014). Despite good physical properties for all EO-NEs, only garlic-NE showed high insecticidal activity (>90 % mortality) against the target pest in preliminary trials.

Few studies investigated EO-NEs on Tephritidae pests. Giunti et al. (2022b) tested anise, fennel, and mint-NEs against *Bactrocera oleae* Rossi (Diptera: Tephritidae), finding ovideterrent effects but no contact toxicity. In contrast, our garlic-NE showed strong ingestion toxicity against Medfly with LD_{50} and LD_{90} of 0.96 % and 2.18 % EO, respectively. While EO-NE efficacy against Tephritidae is poorly explored, the toxicity of EOs in raw form is well documented. Several authors reported the biological activity of EOs against Medfly (Benelli et al., 2013; Miguel et al., 2010; Passino et al., 1999). For instance, Medflies fed with 5 % of *Thymus herba-barona* Loisel and *Cinnamomum zeylanicum* Blume EOs showed >90 % mortality, whereas *Salvia officinalis* L. and *R. officinalis* EOs induced <50 % mortality (Moretti et al., 1998). Conversely, Benelli et al. (2012) reported 100 % mortality with *R. officinalis* at 2.5 %. Similarly, EOs extracted from *Eucalyptus campaspe* S.Moore and *Eucalyptus torquata* Luehm also showed strong toxicity and repellence against *C. capitata* adults through ingestion, contact, and fumigation (Nenaah et al., 2015).

One of the most interesting aspects related to the use of the botanicals (i.e., EOs) used as such or encapsulated into nano-delivery systems is their presumed safety towards non-target. The knowledge about the harmful effects must be implemented considering different factors such as type of natural substances and nano-formulation, application rate, doses, insect species or route of exposure (Modafferi et al., 2024a; Giunti et al., 2022a). Several studies have explored the use of garlic EO-NEs, assessing their impact on non-target organisms. Modafferi et al. (2024b, 2024c) and Ricupero et al. (2022) investigated the effects of garlic-NEs towards *Cryptolaemus montrouzieri* (Mulsant) (Coleoptera: Coccinellidae), as well as honeybees and tomato plants reporting no significant adverse effects. Similarly, Papanikolaou et al. (2018) showed that a natural pyrethrin-based nano-emulsion was effective against *Aphis gossypii* (Glover) (Hemiptera: Aphididae), whereas it did not show toxicity towards two aphid predators, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) and *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae). New nano-formulations may affect non-target organisms, contrary to some authors' findings. For example, Garlic and mandarin NEs were phytotoxic to pepper plants and exhibited lethal and

sub-lethal effects towards *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae), respectively (Campolo et al., 2020b; Giuliano et al., 2024). Our results showed that garlic-NE was highly toxic to *A. daci*, with both tested doses (LD_{50}: 0.96 % EO; LD_{90}: 2.18 % EO) causing 100 % mortality. However, since *C. capitata* is typically targeted using bait formulations (Giunti et al., 2023b), which are more selective towards natural enemies (Urbaneja et al., 2009), and garlic-NE was also tested in bait form, future studies should assess its selectivity in such contexts.

Essential oils are known to interfere with the insect nervous system through multiple mechanisms, including modulation of octopamine and GABA receptors, alteration of calcium ion channels, and inhibition of acetylcholinesterase (AChE) and ATPase activity (Campolo et al., 2018; Enan, 2001; Kostyukovsky et al., 2002; Wang and Heinbockel, 2018). However, gene expression analysis (AChE, TBH, GABAT) showed no significant changes across treatments (C-, LD_{50}, LD_{90}), suggesting that garlic-NE had no direct neurotoxic effects, unlike conventional neurotoxic insecticides. Instead, transcriptional analyses indicated a significant upregulation of genes involved in Phase I (cytochrome P450 monooxygenases) and Phase II (glutathione S-transferases) detoxification pathways, pointing to a metabolic detoxification response as the primary mode of action.

Among Phase I detoxification genes, *Cyp6a2, Cyp6a69,* and *Cyp6g2* were significantly upregulated, with *Cyp6a69* showing ~2-fold upregulation of the LD_{50} and LD_{90} treatments, compared to C- treatment. These genes are widely associated with xenobiotic metabolism and insecticide resistance, playing a key role in oxidative detoxification and metabolic adaptation to toxic compounds (Akami et al., 2019a). Similarly, the increased expression of *Adh* suggests a metabolic response to volatile alcohol-derived compounds present in garlic-NE. Regarding Phase II detoxification, *GSTS1* was significantly overexpressed at LD_{90}, indicating that glutathione conjugation plays a role in neutralizing and excreting garlic-NE-derived metabolites. Previous studies demonstrate that plant-based bioinsecticides induce glutathione S-transferases (GSTs) in insects, as seen in *T. castaneum* larvae exposed to *Artemisia vulgaris* L. EO, where multiple GST genes were overexpressed (Gao et al., 2020). These findings underscore a key difference between EOs and conventional insecticides. While organophosphates, carbamates, and neonicotinoids act via neurotoxicity (Abdelgaleil et al., 2009; Casida, 2018; Kostyukovsky et al., 2002), garlic-NE triggers broad metabolic detoxification. Its toxicity may result from over-stimulation of detox pathways, causing toxic build-up and oxidative stress. This multi-pathway activation could be advantageous for resistance management. Conventional insecticides often target a single metabolic or neural pathway, facilitating the development of resistance in pest populations. Garlic-NE stimulates insects to activate multiple detox pathways simultaneously due to its complex bioactive composition, making resistance development less likely. This supports EOs and their nanoemulsions as promising sustainable alternatives for IPM strategies.

The insect gut microbiome plays a key role in various physiological processes, including digestion, immune system regulation, and detoxification of xenobiotics, which can influence pesticide resistance (Douglas, 2015; Engel and Moran, 2013). Specific gut bacteria can degrade toxic compounds, aiding insecticide metabolism (Kikuchi et al., 2012; Xia et al., 2018). In Medfly, specific bacterial taxa, especially Enterobacteriaceae, support development, nutrition, and possibly behavior, and have been linked to insecticide resistance through xenobiotic metabolism, as seen in *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) (Cheng et al., 2017). In agreement with previous studies, our results confirmed that Enterobacteriaceae represents a dominant taxon in the gut microbiome of *C. capitata*, (Malacrinò et al., 2018; Mason et al., 2023), with *Pluralibacter* spp. being the most abundant genus across all treatments. After excluding Pluralibacter, clear shifts in gut bacterial composition were observed across treatments, indicating that garlic-NE selectively alters microbiome structure. At 24 h, the LD_{30} group showed higher levels of Erwiniaceae, Sphingomonadaceae, and Halomonadaceae, while Enterococcaceae and Pseudomonadaceae dominated in the C- group, and Rhizobiaceae and Halomonadaceae in the Ct group. This suggests an early microbial shift likely driven by garlic-NE's antimicrobial activity. At 72 h, beta diversity analysis revealed a more pronounced restructuring. In the LD_{30} group, Staphylococcaceae and Moraxellaceae increased, while Enterococcaceae and Halomonadaceae remained dominant in controls. These microbial shifts, particularly the reduced presence of Pseudomonadaceae and Enterococcaceae, may impair bacterial-mediated detoxification, supporting the hypothesis that garlic-NE progressively disrupts microbial homeostasis.

The antimicrobial properties of EOs and their nanoemulsions against insect-associated bacteria have been well documented (Dehghankar et al., 2021; Szczepanik et al., 2018). The observed shifts in microbial community structure suggest that garlic-NE selectively modulates gut bacterial populations, potentially altering microbiome functionality. Given the role of certain gut bacteria in xenobiotic metabolism, these microbial changes may compromise the insect's ability to detoxify garlic-NE components. The loss of key microbial taxa could weaken the insect's overall physiological resilience, increasing its susceptibility to the treatment.

Gut bacteria contribute to xenobiotic metabolism and nutrient assimilation, disrupting this balance may impair the insect's detoxification capacity and compromise physiological homeostasis. This dual impact—metabolic disruption combined with microbiome restructuring—suggests a multifaceted mode of action, potentially reducing the likelihood of resistance development by targeting multiple physiological pathways simultaneously (Dada et al., 2018; Gusmão et al., 2010).

Overall, our results indicate that garlic-NE disrupts the gut microbiome of *C. capitata*, with microbial shifts becoming more pronounced over time. This restructuring may contribute to the insecticidal effects of garlic-NE, either by interfering with microbial-mediated detoxification processes or by inducing gut dysbiosis, which could impact host fitness and survival. Disrupting microbial homeostasis could profoundly affect insect physiology, particularly in metabolic processes associated with xenobiotic degradation. While our study demonstrates that garlic-NE induces structural changes in the microbiota, future research should assess the functional consequences of these shifts, particularly their impact on enzymatic pathways related to insecticide detoxification.

5. Conclusion

This study highlights the potential of EO-NEs as innovative tools for pest management, offering an alternative to synthetic insecticides. The successful application of microfluidization demonstrates its effectiveness in enhancing EO stability, overcoming key limitations such as high volatility and poor solubility. The findings suggest that garlic-NE exerts its insecticidal action through metabolic disruption rather than direct neurotoxicity, activating multiple detoxification pathways and altering the gut microbiota of *C. capitata*. This multifaceted mode of action could reduce the likelihood of resistance development, a major concern in pest control. Our findings indicate that garlic-NE toxicity in *C. capitata* is not solely attributed to its direct insecticidal effects but also to its ability to disrupt key metabolic pathways and alter the gut microbiota. These microbiome shifts may impair detoxification efficiency, increasing susceptibility to toxic stressors. Understanding this complex interaction between metabolic detoxification and microbiota modulation provides new insights into the mode of action of EO-NEs and highlights their potential as sustainable alternatives for pest management. However, the impact of garlic-NE on non-target organisms, particularly beneficial insects, raises concerns about its selectivity and suitability for Integrated Pest Management (IPM) strategies. Future research should minimize off-target effects, evaluate long-term ecological consequences, and optimize its use in real-world agricultural settings. These efforts will be essential to support the use of EO-NEs in sustainable pest management programs and to ensure their effectiveness, environmental compatibility, and practical applicability at a commercial scale.

CRediT authorship contribution statement

Antonino Modafferi: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Alberto Urbaneja:** Writing – review & editing, Funding acquisition, Formal analysis, Conceptualization. **Cristina M. Aure:** Investigation. **Giulia Giunti:** Writing – review & editing, Formal analysis, Data curation. **Isabel Martinez-Sañudo:** Investigation, Formal analysis. **Annamaria Ienco:** Investigation. **Orlando Campolo:** Writing – review & editing, Formal analysis, Conceptualization. **Vincenzo Palmeri:** Writing – review & editing, Funding acquisition. **Meritxell Perez-Hedo:** Writing – review & editing, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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 article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pestbp.2025.106569.

Data availability

Data will be made available on request.

References

Abad-Moyano, R., Pina, T., Ferragut, F., Urbaneja, A., 2009. Comparative life-history traits of three phytoseiid mites associated with *Tetranychus urticae* (Acari: Tetranychidae) colonies in clementine orchards in eastern Spain: implications for biological control. Exp. Appl. Acarol. 47. https://doi.org/10.1007/s10493-008-9197-z.

Abbott, W.S., 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265.

Abdelgaleil, S.A.M., Mohamed, M.I.E., Badawy, M.E.I., El-Arami, S.A.A., 2009. Fumigant and contact toxicities of monoterpenes to *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) and their inhibitory effects on acetylcholinesterase activity. J. Chem. Ecol. 35. https://doi.org/10.1007/s10886-009-9635-3.

Adams, R.P., 2017. Identification of Essential Oil Components by Gas Chromatography/ Mass Spectrometry. 5, online ed. Texensis Publishing, Gruver, TX USA.

Akami, M., Njintang, N.Y., Gbaye, O., Niu, C.Y., Nukenine, E.N., 2019a. Comparative expression of two detoxification genes by *Callosobruchus maculatus* in response to dichlorvos and Lippia adoensis essential oil treatments. J. Pest. Sci. 92. https://doi.org/10.1007/s10340-018-01075-4.

Akami, M., Njintang, N.Y., Gbaye, O.A., Andongma, A.A., Rashid, M.A., Niu, C.Y., Nukenine, E.N., 2019b. Gut bacteria of the cowpea beetle mediate its resistance to dichlorvos and susceptibility to *Lippia adoensis* essential oil. Sci. Rep. 9. https://doi.org/10.1038/s41598-019-42843-1.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26. https://doi.org/10.1046/j.1442-9993.2001.01070.x.

Anderson, M.J., 2006. Distance-based tests for homogeneity of multivariate dispersions. Biometrics 62. https://doi.org/10.1111/j.1541-0420.2005.00440.x.

Anderson, M.J., Walsh, D.C.I., 2013. PERMANOVA, ANOSIM, and the mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing? Ecol. Monogr. 83. https://doi.org/10.1890/12-2010.1.

Benelli, G., Flamini, G., Canale, A., Cioni, P.L., Conti, B., 2012. Toxicity of some essential oil formulations against the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae). Crop Prot. 42, 223–229. https://doi.org/10.1016/J.CROPRO.2012.05.024.

Benelli, G., Canale, A., Flamini, G., Cioni, P.L., Demi, F., Ceccarini, L., Macchia, M., Conti, B., 2013. Biotoxicity of *Melaleuca alternifolia* (Myrtaceae) essential oil against the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), and its parasitoid *Psyttalia concolor* (Hymenoptera: Braconidae). Ind. Crop. Prod. 50, 596–603. https://doi.org/10.1016/J.INDCROP.2013.08.006.

Blenau, W., Rademacher, E., Baumann, A., 2012. Plant essential oils and formamidines as insecticides/ acaricides: what are the molecular targets? Apidologie 43. https://doi.org/10.1007/s13592-011-0108-7.

Blythe, E.K., Tabanca, N., Demirci, B., Kendra, P.E., 2020. Chemical composition of essential oil from *Tetradenia riparia* and its attractant activity for Mediterranean fruit fly, *Ceratitis capitata*. Nat. Prod. Commun. 15. https://doi.org/10.1177/1934578X20953955.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13. https://doi.org/10.1038/nmeth.3869.

Callahan, B.J., McMurdie, P.J., Holmes, S.P., 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J. 11. https://doi.org/10.1038/ismej.2017.119.

Campolo, O., 2022. Essential oil-based green nano-insecticides: formulation, biological activity and application in ecofriendly pest control strategies. Acta Hortic. 1354. https://doi.org/10.17660/ActaHortic.2022.1354.22.

Campolo, O., Giunti, G., Russo, A., Palmeri, V., Zappalà, L., 2018. Essential oils in stored product insect pest control. J. Food Qual. https://doi.org/10.1155/2018/6906105.

Campolo, O., Giunti, G., Laigle, M., Michel, T., Palmeri, V., 2020a. Essential oil-based nano-emulsions: effect of different surfactants, sonication and plant species on physicochemical characteristics. Ind. Crop. Prod. 157. https://doi.org/10.1016/j.indcrop.2020.112935.

Campolo, O., Puglisi, I., Barbagallo, R.N., Cherif, A., Ricupero, M., Biondi, A., Palmeri, V., Baglieri, A., Zappalà, L., 2020b. Side effects of two citrus essential oil formulations on a generalist insect predator, plant and soil enzymatic activities. Chemosphere 257. https://doi.org/10.1016/j.chemosphere.2020.127252.

Casida, J.E., 2018. Neonicotinoids and other insect nicotinic receptor competitive modulators: Progress and prospects. Annu. Rev. Entomol. 63. https://doi.org/10.1146/annurev-ento-020117-043042.

Cheng, D., Guo, Z., Riegler, M., Xi, Z., Liang, G., Xu, Y., 2017. Gut symbiont enhances insecticide resistance in a significant pest, the oriental fruit fly *Bactrocera dorsalis* (Hendel). Microbiome 5. https://doi.org/10.1186/s40168-017-0236-z.

Chueca, P., Montón, H., Ripollés, J.L., Castañera, P., Moltó, E., Urbaneja, A., 2007. Spinosad bait treatments as alternative to malathion to control the Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae) in the Mediterranean Basin. J. Pestic. Sci. 32. https://doi.org/10.1584/jpestics.G07-26.

Dada, N., Sheth, M., Liebman, K., Pinto, J., Lenhart, A., 2018. Whole metagenome sequencing reveals links between mosquito microbiota and insecticide resistance in malaria vectors. Sci. Rep. 8. https://doi.org/10.1038/s41598-018-20367-4.

Davies, N.W., 1990. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and Carbowax 20M phases. J. Chromatogr. A 503, 1–24. https://doi.org/10.1016/S0021-9673(01)81487-4.

de Pedro, L., Beitia, F., Sabater-Muñoz, B., Asís, J.D., Tormos, J., 2016. Effect of temperature on the developmental time, survival of immatures and adult longevity of *Aganaspis daci* (Hymenoptera: Figitidae), a natural enemy of *Ceratitis capitata* (Diptera: Tephritidae). Crop Prot. 85. https://doi.org/10.1016/j.cropro.2016.03.010.

de Pedro, L., Tormos, J., Asís, J.D., Sabater-Muñoz, B., Beitia, F., 2018. Biology of *Aganaspis daci* (Hymenoptera: Figitidae), parasitoid of *Ceratitis capitata* (Diptera: Tephritidae): mode of reproduction, biological parameters and superparasitism. Crop Prot. 108. https://doi.org/10.1016/j.cropro.2018.02.015.

Dehghankar, M., Maleki-Ravasan, N., Tahghighi, A., Karimian, F., Karami, M., 2021. Bioactivities of rose-scented geranium nanoemulsions against the larvae of *Anopheles stephensi* and their gut bacteria. PLoS One 16. https://doi.org/10.1371/journal.pone.0246470.

Donsì, F., Ferrari, G., 2016. Essential oil nanoemulsions as antimicrobial agents in food. J. Biotechnol. 233, 106–120. https://doi.org/10.1016/J.JBIOTEC.2016.07.005.

Douglas, A.E., 2015. Multiorganismal insects: diversity and function of resident microorganisms. Annu. Rev. Entomol. 60. https://doi.org/10.1146/annurev-ento-010814-020822.

Enan, E., 2001. Insecticidal activity of essential oils: Octopaminergic sites of action. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 130. https://doi.org/10.1016/S1532-0456(01)00255-1.

Engel, P., Moran, N.A., 2013. The gut microbiota of insects-diversity in structure and function. FEMS Microbiol. Rev. 37. https://doi.org/10.1111/1574-6976.12025.

Gao, S., Zhang, K., Wei, L., Wei, G., Xiong, W., Lu, Y., Zhang, Y., Gao, A., Li, B., 2020. Insecticidal activity of *Artemisia vulgaris* essential oil and transcriptome analysis of *Tribolium castaneum* in response to oil exposure. Front. Genet. 11. https://doi.org/10.3389/fgene.2020.00589.

Garcia, F.R.M., Ovruski, S.M., Suárez, L., Cancino, J., Liburd, O.E., 2020. Biological control of tephritid fruit flies in the Americas and Hawaii: a review of the use of parasitoids and predators. Insects 11. https://doi.org/10.3390/insects11100662.

Giuliano, G., Campolo, O., Forte, G., Urbaneja, A., Pérez-Hedo, M., Latella, I., Palmeri, V., Giunti, G., 2024. Insecticidal activity of *Allium sativum* essential oil-based nanoemulsion against *Spodoptera littoralis*. Insects 15, 476. https://doi.org/10.3390/INSECTS15070476/S1.

Giunti, G., Palermo, D., Laudani, F., Algeri, G.M., Campolo, O., Palmeri, V., 2019. Repellence and acute toxicity of a nano-emulsion of sweet orange essential oil toward two major stored grain insect pests. Ind. Crop. Prod. 142, 111869. https://doi.org/10.1016/j.indcrop.2019.111869.

Giunti, G., Benelli, G., Palmeri, V., Laudani, F., Ricupero, M., Ricciardi, R., Maggi, F., Lucchi, A., Guedes, R.N.C., Desneux, N., Campolo, O., 2022a. Non-target effects of essential oil-based biopesticides for crop protection: impact on natural enemies, pollinators, and soil invertebrates. Biol. Control 176, 105071. https://doi.org/10.1016/J.BIOCONTROL.2022.105071.

Giunti, G., Laudani, F., Lo Presti, E., Bacchi, M., Palmeri, V., Campolo, O., 2022b. Contact toxicity and ovideterrent activity of three essential oil-based nano-emulsions against the olive fruit fly *Bactrocera oleae*. Horticulturae 8. https://doi.org/10.3390/horticulturae8030240.

Giunti, G., Benelli, G., Campolo, O., Canale, A., Kapranas, A., Liedo, P., De Meyer, M., Nestel, D., Ruiu, L., Scolari, F., Wang, X., Papadopoulos, N.T., 2023a. Biology, ecology and invasiveness of the Mediterranean fruit fly, *Ceratitis capitata*: a review. Entomol. Gen. 43. https://doi.org/10.1127/entomologia/2023/2135.

Giunti, G., Benelli, G., Campolo, O., Canale, A., Kapranas, A., Liedo, P., De Meyer, M., Nestel, D., Ruiu, L., Scolari, F., Wang, X., Papadopoulos, N.T., 2023b. Management of the Mediterranean fruit fly, *Ceratitis capitata*: past, present, and future. Entomol. Gen 43. https://doi.org/10.1127/entomologia/2023/2136.

Gusmão, D.S., Santos, A.V., Marini, D.C., Bacci, M., Berbert-Molina, M.A., Lemos, F.J.A., 2010. Culture-dependent and culture-independent characterization of microorganisms associated with *Aedes aegypti* (Diptera: Culicidae) (L.) and dynamics of bacterial colonization in the midgut. Acta Trop. 115. https://doi.org/10.1016/j.actatropica.2010.04.011.

Hassan, M.E., Mohafrash, S.M.M., Mikhail, M.W., Mossa, A.T.H., 2023. Development and evaluation of clove and cinnamon oil-based nanoemulsions against adult fleas (*Xenopsylla cheopis*). Biocatal. Agric. Biotechnol. 47. https://doi.org/10.1016/j.bcab.2022.102587.

Hendrichs, J., Katsoyannos, B.I., Wornoayporn, V., Hendrichs, M.A., 1994. Odour-mediated foraging by yellowjacket wasps (Hymenoptera: Vespidae): predation on leks of pheromone-calling Mediterranean fruit fly males (Diptera: Tephritidae). Oecologia 99. https://doi.org/10.1007/BF00317087.

Jacas Miret, J.A., Palou, L., Beitia, F., Del Río, M.A., 2008. Controlled in vivo infestation of mandarin fruit with *Ceratitis capitata* for development of quarantine treatments. Span. J. Agric. Res. 6. https://doi.org/10.5424/sjar/2008063-336.

Jankowska, M., Rogalska, J., Wyszkowska, J., Stankiewicz, M., 2018. Molecular targets for components of essential oils in the insect nervous system—a review. Molecules 23. https://doi.org/10.3390/molecules23010034.

Jennings, W., 2012. Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography. Elsevier.

Juan-Blasco, M., Sabater-Muñoz, B., Argilés, R., Jacas, J.A., Ortego, F., Urbaneja, A., 2013. Effects of pesticides used on citrus grown in Spain on the mortality of *Ceratitis capitata* (Diptera: Tephritidae) Vienna-8 strain sterile males. J. Econ. Entomol. 106. https://doi.org/10.1603/EC12464.

Kikuchi, Y., Hayatsu, M., Hosokawa, T., Nagayama, A., Tago, K., Fukatsu, T., 2012. Symbiont-mediated insecticide resistance. Proc. Natl. Acad. Sci. USA 109. https://doi.org/10.1073/pnas.1200231109.

Kostyukovsky, M., Rafaeli, A., Gileadi, C., Demchenko, N., Shaaya, E., 2002. Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: possible mode of action against insect pests. Pest Manag. Sci. 58. https://doi.org/10.1002/ps.548.

Koul, O., 2019. Nanobiopesticides: An introduction. Nano-Biopestic. Today Fut. Perspect. 1–15. https://doi.org/10.1016/B978-0-12-815829-6.00001-2.

Laudani, F., Campolo, O., Caridi, R., Latella, I., Modafferi, A., Palmeri, V., Sorgonà, A., Zoccali, P., Giunti, G., 2022. Aphicidal activity and phytotoxicity of *Citrus sinensis* essential-oil-based nano-insecticide. Insects 13, 1150. https://doi.org/10.3390/INSECTS13121150.

Liquido, N.J., Cunningham, R.T., Nakagawa, S., 1990. Host plants of Mediterranean fruit fly (Diptera: Tephritidae) on the island of Hawaii (1949-1985 survey). J. Econ. Entomol. 83. https://doi.org/10.1093/jee/83.5.1863.

Malacrinò, A., Campolo, O., Medina, R.F., Palmeri, V., 2018. Instar- and host-associated differentiation of bacterial communities in the Mediterranean fruit fly *Ceratitis capitata*. PLoS One 13. https://doi.org/10.1371/journal.pone.0194131.

Masada, Y., 1976. Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry. John Wiley & Sons, New York.

Mason, C.J., Auth, J., Geib, S.M., 2023. Gut bacterial population and community dynamics following adult emergence in pest tephritid fruit flies. Sci. Rep. 13. https://doi.org/10.1038/s41598-023-40562-2.

Mavrikakis, P.G., Economopoulos, A.P., Carey, J.R., 2000. Continuous winter reproduction and growth of the Mediterranean fruit fly (Diptera: tephritidae) in Heraklion, Crete, southern Greece. Environ. Entomol. 29. https://doi.org/10.1603/0046-225x-29.6.1180.

McLafferty, F.W., Stauffer, D.B., Stenhagen, E., Heller, S.R., 1989. The Wiley/NBS registry of mass spectral data. J. Chem. Educ. 66, 256.

Miguel, M.G., Almeida, M.L., Gonçalves, M.A., Figueiredo, A.C., Barroso, J.G., Pedro, L. M., 2010. Toxic effects of three essential oils on *Ceratitis capitata*. J. Essent. Oil-Bear. Plants 13. https://doi.org/10.1080/0972060X.2010.10643811.

Modafferi, A., Giunti, G., Benelli, G., Campolo, O., 2024a. Ecological costs of botanical nano-insecticides. Curr. Opin. Environ. Sci. Health 100579. https://doi.org/10.1016/J.COESH.2024.100579.

Modafferi, A., Giunti, G., Urbaneja, A., Laudani, F., Latella, I., Pérez-Hedo, Meritxell, Ricupero, M., Palmeri, V., Campolo, Orlando, 2024b. High-energy emulsification of *Allium sativum* essential oil boosts insecticidal activity against *Planococcus citri* with no risk to honeybees. J. Pest. Sci. 1–12. https://doi.org/10.1007/S10340-024-01800-2.

Modafferi, A., Ricupero, M., Mostacchio, G., Latella, I., Zappalà, L., Palmeri, V., Garzoli, S., Giunti, G., Campolo, O., 2024c. Bioactivity of *Allium sativum* essential oil-based nano-emulsion against *Planococcus citri* and its predator *Cryptolaemus montrouzieri*. Ind. Crop. Prod. 208, 117837. https://doi.org/10.1016/J.INDCROP.2023.117837.

Modafferi, A., Urbaneja, A., Aure, C.M., Laudani, F., Palmeri, V., Giunti, G., et al., 2025. Green pest control strategies: essential oil-based nano-emulsions for *Delottococcus aberiae* management. J. Pest. Sci. 98, 1263–1275. https://doi.org/10.1007/s10340-025-01914-1.

Mohammed, T.G.M., Nasr, M.E.H., 2020. Preparation, characterization and biological efficacy of eucalyptus oil nanoemulsion against the stored grain insects. Asian J. Agric. Res. https://doi.org/10.9734/ajaar/2020/v13i230102.

Moretti, M.D.L., Bazzoni, E., Passino, G.S., Prota, R., 1998. Antifeedant effects of some essential oils on *Ceratitis capitata* Wied. (Diptera, Tephritidae). J. Essent. Oil Res. 10. https://doi.org/10.1080/10412905.1998.9700930.

Mossa, A.T.H., 2016. Green pesticides: essential oils as biopesticides in insect-pest management. J. Environ. Sci. Technol. 9. https://doi.org/10.3923/jest.2016.354.378.

Moustafa, H., Mohamad, T., Torkey, H., et al., 2015. Effect of formulated nanoemulsion of eucalyptus oil on the cotton bollworms. J. Biol. Chem. Res 32.

Nenaah, G.E., Ibrahim, S.I.A., Al-Assiuty, B.A., 2015. Chemical composition, insecticidal activity and persistence of three Asteraceae essential oils and their nanoemulsions against *Callosobruchus maculatus* (F.). J. Stored Prod. Res. 61. https://doi.org/10.1016/j.jspr.2014.12.007.

Nirmala, M.J., Dhas, S.P., Saikrishna, N., Raj, U.S., Sai, P.S., Nagarajan, R., 2022. Green nanoemulsions: components, formulation, techniques of characterization, and applications. Bio-Based Nanoemuls. Agri-Food Applicat. https://doi.org/10.1016/B978-0-323-89846-1.00013-9.

Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., et al., 2015. Ordination Methods, Diversity Analysis and Other Functions for Community and Vegetation Ecologists. Community Ecology Package, Vegan.

Ortolá, B., Urbaneja, A., Eiras, M., Pérez-Hedo, M., Daròs, J.A., 2024. RNAi-mediated silencing of Mediterranean fruit fly (*Ceratitis capitata*) endogenous genes using orally-supplied double-stranded RNAs produced in Escherichia coli. Pest Manag. Sci. 80. https://doi.org/10.1002/ps.7839.

Papachristos, D.P., Kimbaris, A.C., Papadopoulos, N.T., Polissiou, M.G., 2009. Toxicity of citrus essential oils against *Ceratitis capitata* (Diptera: Tephritidae) larvae. Ann. Appl. Biol. 155. https://doi.org/10.1111/j.1744-7348.2009.00350.x.

Papanikolaou, N.E., Kalaitzaki, A., Karamaouna, F., Michaelakis, A., Papadimitriou, V., Dourtoglou, V., et al., 2018. 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. 25. https://doi.org/10.1007/s11356-017-8596-2.

Park, C.G., Jang, M., Yoon, K.A., Kim, J., 2016. Insecticidal and acetylcholinesterase inhibitory activities of Lamiaceae plant essential oils and their major components against *Drosophila suzukii* (Diptera: Drosophilidae). Ind. Crop. Prod. 89. https://doi.org/10.1016/j.indcrop.2016.06.008.

Passino, G.S., Bazzoni, E., Moretti, M.D.L., Prota, R., 1999. Effects of essential oil formulations on *Ceratitis capitata* Wied. (Dipt., Tephritidae) adult flies. J. Appl. Entomol. 123, 45–149. https://doi.org/10.1046/J.1439-0418.1999.00332.X.

Pavoni, L., Perinelli, D.R., Bonacucina, G., Cespi, M., Palmieri, G.F., 2020. An overview of micro- and nanoemulsions as vehicles for essential oils: formulation, preparation and stability. Nanomaterials 135 (10), 135. https://doi.org/10.3390/NANO10010135.

Ponton, F., Chapuis, M.P., Pernice, M., Sword, G.A., Simpson, S.J., 2011. Evaluation of potential reference genes for reverse transcription-qPCR studies of physiological responses in *Drosophila melanogaster*. J. Insect Physiol. 57, 840–850. https://doi.org/10.1016/J.JINSPHYS.2011.03.014.

Popescu, I.E., Gostin, I.N., Blidar, C.F., 2024. An overview of the mechanisms of action and administration technologies of the essential oils used as green insecticides. AgriEngineering 6 (2), 1195–1217. https://doi.org/10.3390/agriengineering6020068.

Priestley, C.M., Williamson, E.M., Wafford, K.A., Sattelle, D.B., 2003. Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human GABA (a) receptors and a homo-oligomeric GABA receptor from *Drosophila melanogaster*. Br. J. Pharmacol. 140, 1363–1372. https://doi.org/10.1038/SJ.BJP.0705542.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41. https://doi.org/10.1093/nar/gks1219.

Ricupero, M., Biondi, A., Cincotta, F., Condurso, C., Palmeri, V., Verzera, A., Zappalà, L., Campolo, O., 2022. Bioactivity and physico-chemistry of garlic essential oil nanoemulsion in tomato. Entomol. Gen. 42, 921–930. https://doi.org/10.1127/entomologia/2022/1553.

Ricupero, M., Biondi, A., Campolo, O., Alonso-Valiente, M., Gallego, C., Zappalà, L., Urbaneja, A., Pérez-Hedo, M., 2023. Garlic and peppermint essential oils elicit plant defensive responses in sweet peppers. Entomol. Gen. 43, 1001–1010. https://doi.org/10.1127/ENTOMOLOGIA/2023/2115.

Seo, S.M., Kim, J., Kang, J., Koh, S.H., Ahn, Y.J., Kang, K.S., Park, I.K., 2014. Fumigant toxicity and acetylcholinesterase inhibitory activity of 4 Asteraceae plant essential

oils and their constituents against Japanese termite (*Reticulitermes speratus* Kolbe). Pestic. Biochem. Physiol. 113. https://doi.org/10.1016/j.pestbp.2014.06.001.

Shaaya, E., Rafaeli, A., 2007. Essential oils as biorational insecticides-potency and mode of action. Insecticid. Design Using Adv. Technol. https://doi.org/10.1007/978-3-540-46907-0_11.

Sugumar, S., Clarke, S.K., Nirmala, M.J., Tyagi, B.K., Mukherjee, A., Chandrasekaran, N., 2014. Nanoemulsion of eucalyptus oil and its larvicidal activity against *Culex quinquefasciatus*. Bull. Entomol. Res. 104, 393–402. https://doi.org/10.1017/S0007485313000710.

Szczepanik, M., Walczak, M., Zawitowska, B., Michalska-Sionkowska, M., Szumny, A., Wawrzeńczyk, C., Brzezinska, M.S., 2018. Chemical composition, antimicromicrobial activity and insecticidal activity against the lesser mealworm *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) of *Origanum vulgare* L. ssp. hirtum (Link) and *Artemisia dracunculus* L. essential oils. J. Sci. Food Agric. 98. https://doi.org/10.1002/jsfa.8524.

Urbaneja, A., Marí, F.G., Tortosa, D., Navarro, C., Vanaclocha, P., Bargues, L., Castañera, P., 2006. Influence of ground predators on the survival of the Mediterranean fruit fly pupae, *Ceratitis capitata*, in Spanish citrus orchards. BioControl 51. https://doi.org/10.1007/s10526-005-2938-6.

Urbaneja, A., Chueca, P., Montón, H., Pascual-Ruiz, S., Dembilio, O., Vanaclocha, P., Abad-Moyano, R., Pina, T., Castañera, P., 2009. Chemical alternatives to malathion for controlling *Ceratitis capitata* (Diptera: Tephritidae), and their side effects on natural enemies in Spanish citrus orchards. J. Econ. Entomol. 102. https://doi.org/10.1603/029.102.0121.

Van Den Dool, Kratz, P.D., 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr. https://doi.org/10.1016/S0021-9673(01)80947-X.

Wang, Z.J., Heinbockel, T., 2018. Essential oils and their constituents targeting the gabaergic system and sodium channels as treatment of neurological diseases. Molecules 23. https://doi.org/10.3390/molecules23051061.

Wickham, Hadley, 2009. Ggplot2 : elegant graphics for data analysis. J. Stat. Softw. 35.

Xia, X., Sun, B., Gurr, G.M., Vasseur, L., Xue, M., You, M., 2018. Gut microbiota mediate insecticide resistance in the diamondback moth, *Plutella xylostella* (L.). Front. Microbiol. 9. https://doi.org/10.3389/fmicb.2018.00025.

Xu, S., Zhan, L., Tang, W., Wang, Q., Dai, Z., Zhou, L., Feng, T., Chen, M., Wu, T., Hu, E., Yu, G., 2023. MicrobiotaProcess: a comprehensive R package for deep mining microbiome. Innovation 4. https://doi.org/10.1016/j.xinn.2023.100388.

Yeom, H.J., Jung, C.S., Kang, J., Kim, J., Lee, J.H., Kim, D.S., Kim, H.S., Park, P.S., Kang, K.S., Park, I.K., 2015. Insecticidal and acetylcholine esterase inhibition activity of asteraceae plant essential oils and their constituents against adults of the German cockroach (*Blattella germanica*). J. Agric. Food Chem. 63. https://doi.org/10.1021/jf505927n.

Zhou, C., Rao, Yong, Rao, Yi, 2008. A subset of octopaminergic neurons are important for *Drosophila* aggression. Nat. Neurosci. 11, 1059–1067. https://doi.org/10.1038/nn.2164.