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Allium sativum essential oil: Different susceptibility of Diptera larvae and adults to its arsenal of sulfur derivatives

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ABSTRACT

Essential oils (EOs) have varied compositions depending on several intrinsic and extrinsic factors. Despite being generally indicated as valuable insecticides, the prevalence of a chemical class can determine an EO's more or less marked bioactivity. For this purpose, we selected an *Allium sativum* L. EO rich in sulfur derivatives, *Cinnamomum verum* J.Presl EO characterized by phenylpropanoids, *Origanum vulgare* L. EO chiefly composed of oxygenated monoterpenes, and *Ruta graveolens* L. EO abundant in non-terpene derivatives. The complete composition of the EOs was ascertained through gas chromatography-electron impact mass spectrometry. As insect pests of economic relevance, we focused on the Diptera the blue bottle fly *Calliphora vomitoria* (Linnaeus) (Calliphoridae) and the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Tephritidae). The mean lethal concentrations (LC_{50}) by fumigation and mean lethal doses (LD_{50}) by direct contact of the four EOs on adult specimens and wandering larvae were evaluated. The *A. sativum* EO was identified as the only one able to control both developmental stages of the target pests. Besides highlighting a different susceptibility between the two flies, with *Ca. vomitoria* being less susceptible (susceptibility ratios – SR from 1.37 to 26.29), we also observed a distinctly lower susceptibility of the wandering larvae compared to their respective adults (SR with the *A. sativum* EO of 15.64 by fumigation and 156.17 by contact for *Ca. vomitoria*; 72.42 and 107.29, respectively for *Ce. capitata*). Therefore, species-specific susceptibility of insects, regardless of body size but related to ontogeny and different levels of detoxifying enzymes possessed, can be hypothesized.

1. Introduction

Essential oils (EOs) are heterogeneous mixtures of secondary metabolites obtained through distillation (water- or steam-mediated) or mechanical processes from a plethora of medicinal and aromatic plants belonging to dozens of botanical families. On average, an EO comprises 20–60 constituents, usually with just a few prevailing. The composition radically varies mainly according to the employed vegetal species and organs (*e.g.*, leaves, flowers, fruits, peels, seeds, bark, roots) but also plant growth parameters (*e.g.*, season, climate, altitude, temperature, sun exposure, soil conditions), handling (*e.g.*, agronomic practices, balsamic time, harvesting, drying, distillation modalities), and genetic factors affecting the secondary metabolism (Nabi et al., 2025; Jyotsna et al., 2024).

Chemically speaking, the main classes of compounds detected in EOs are terpenes, phenylpropanoids, non-terpene derivatives, and sulfur derivatives (Zuzarte and Salgueiro, 2015). Monoterpenes are terpenes made of two isoprene units and represent the most abundant class of plant secondary metabolites. Among others, they include the monoterpene hydrocarbons, made up of only carbon and hydrogen, and oxygenated monoterpenes (monoterpenoids) with an additional oxygen functionality. Phenylpropanoids encompass a six-carbon aromatic phenyl group and a three-carbon propene tail. They are synthesized through the shikimate pathway from the amino acid phenylalanine. Sulfur derivatives contain the chemical element sulfur and, even when found in small amounts in an extract, dominate its perceived smell. EOs also include non-terpene derivatives biogenerated by the phenylpropanoid pathway.

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When talking about the application of EOs as toxic, repellent/deterrent, or attractive agents towards arthropod pests, they are indiscriminately indicated as valid biorational products thanks to their natural origin, fast decomposability, low persistence in the environment, and scarce toxicity on vertebrates (Jyotsna et al., 2024). Anyway, it is more reasonable to think that almost every EO has a more or less marked bioactivity on each organism and developmental stage linked exactly to its own peculiar composition. It was verified that some EO phytoconstituents, regardless of the chemical class to which they belong, can impair the activity of the enzyme acetylcholinesterase, the inhibitory neurotransmitter gamma-aminobutyric acid, or octopamine (Jyotsna et al., 2024; Talepour et al., 2021). Besides the neurotoxic action, EO components can affect enzymes related to detoxification, digestion, metabolism, and chitin synthesis (Adesanya et al., 2018; Deb and Kumar, 2020; Shah et al., 2021; Subaharan et al., 2021), thus generating a multitude of sub-lethal effects (Huang et al., 2000; Chang et al., 2019).

From a literature analysis, we identified four common medicinal and aromatic plants that produce EOs characterized by the prevalence (> 75%) of a specific chemical class. In detail, we selected for our trials the EOs from *Allium sativum* L. (Amaryllidaceae) bulbs, *Cinnamomum verum* J.Presl (Lauraceae) bark, *Origanum vulgare* L. (Lamiaceae) aerial parts, and *Ruta graveolens* L. (Rutaceae) leaves as being rich in sulfur derivatives, phenylpropanoids, oxygenated monoterpenes, and non-terpene derivatives, respectively (Palermo et al., 2021; Aungtikun and Soonwera, 2021; Raal et al., 2024; Lee et al., 2023).

As target insect pests, we focused on adults and wandering (*i.e.*, post-feeding third instars) larvae of two Diptera species of economic relevance: the blue bottle fly *Calliphora vomitoria* (Linnaeus) (Calliphoridae) and the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Tephritidae). Despite Calliphoridae being good pollinators of fruit trees and horticultural crops and key arthropods to estimating the minimum post-mortem interval in medicolegal investigations, *Ca. vomitoria* is sometimes considered a pest (Farina et al., 2026). Indeed, it can be present in urban areas, and besides representing a nuisance, females can lay eggs on raw, processed, and dried meat products, and the developing larvae determine their spoilage. *Ceratitis capitata* is a highly invasive species native to sub-Saharan Africa and a polyphagous threat to wild and commercial plants, with broadly varying hosts depending on the region and season. It is considered the most detrimental fly in the field, primarily for citrus fruits and various stone fruits, berries, peppers, and nuts (OEPP/EPPO, 2011). In non-infested countries, especially China, Japan, New Zealand, and some USA states, *Ce. capitata* is a quarantine pest.

Various EOs with diverse compositions have been tested as toxic, repellent/deterrent, or attractive agents to manage both species, especially their adults. However, a side-by-side evaluation of the bioactivity of EOs based on their prevailing chemical class through relative toxicity (RT) ratios, a direct comparison of ontogenetic susceptibility (wandering larvae *vs*. adults), or the calculation of susceptibility ratios (SR) across developmental stages and species has never been attempted before.

Therefore, the aim of this work was first to determine the median lethal concentrations (LC_{50}) and concentrations lethal for 95% of specimens (LC_{95}) by fumigation and the median lethal doses (LD_{50}) and doses lethal for 95% of specimens (LD_{95}) by direct contact on adult and wandering larvae of *Ca. vomitoria* and *Ce. capitata* when exposed to the four EOs selected for their varied compositions. We opted for wandering larvae as they voluntarily leave the food substrate searching for a dry site for pupation, making them easily recognizable. The median lethal values were used to compare the toxicity level of the EOs, while the LC_{95} and LD_{95} values gave us more practical information on the potential use of such botanical insecticides against the two economically relevant pests. Consequently, based on the obtained results, we identified and discussed the most effective EO among the four involved and the most susceptible Diptera species and developmental stage.

2. Materials and methods

2.1. Essential oils purchase and chemical characterization

The four EOs here employed were purchased from commercial suppliers: *A. sativum* from Giorgini Dr. Martino (Marradi, Italy), *C. verum* from Sigma-Aldrich S.r.l. (Milan, Italy), *O. vulgare* from Herbal Products Italia S.r.l. (Sant'Elena, Italy), and *R. graveolens* from L'Aromoteca (Assago, Italy). They were chosen as all being edible EOs, considering their potential application on food to protect it from the two target Diptera pests.

The EOs were chemically characterized in triplicate ($n = 3$) at the Department of Pharmacy of the University of Pisa. Each EO was diluted to 5% in HPLC-grade *n*-hexane and then injected into a GC–MS apparatus. Gas chromatography–electron impact mass spectrometry (GC–EIMS) analyses were performed with an Agilent 7890B gas chromatograph (Agilent Technologies Incorporated, Santa Clara, CA, USA) equipped with an Agilent HP-5MS (Agilent Technologies Inc.) capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) and an Agilent 5977B single quadrupole mass detector (Agilent Technologies Inc.). Analytical conditions were as follows: injector and transfer line temperatures 220 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at 3 °C min^{-1}; carrier gas helium at 1 mL min^{-1}; injection of 1 μL (5% HPLC grade *n*-hexane solution); split ratio 1:25. Acquisition parameters were as follows: full scan; scan range: 30–300 *m/z*; scan time: 1.0 s.

The identification of the constituents was based on the comparison of their retention times with those of the authentic samples (when available), comparing their linear retention indices relative to the series of *n*-hydrocarbons (C6-C25). Computer matching was also used against the National Institute of Standards and Technology, a laboratory-developed mass spectra library built up from pure substances and components of commercial EOs of known composition, and mass spectrometry literature data.

2.2. Calliphora vomitoria rearing

Calliphora vomitoria larvae were purchased from a commercial supplier of live fishing baits (Nonno Ippei, Vittoria Apuana, Italy). They were reared under laboratory conditions (23 ± 2 °C, 60–65% RH, natural photoperiod) inside polypropylene boxes (27 × 21 × 12 cm) with a netted lid for ventilation and fed beef mince. Species identification was carried out on twenty randomly picked specimens by using the specific keys (Szpila, 2012). When wandering, meaning when they voluntarily left the meat substrate after about four days from the purchase, we used part of the third-instar larvae in the toxicity bioassays and let part of them pupate and then emerge as adults into 75 × 75 × 115 cm knitted mesh and polyester tents (BugDorm-2400 Insect Rearing Tent, MegaView Science Co., Ltd., Taichung, Taiwan). Adults, starting to emerge after one week from the beginning of pupation, were provided with water and a solid diet made of sucrose and yeast extract 4:1 *w/w ad libitum*. Seven-14-day old adults were used in the toxicity bioassays.

2.3. Ceratitis capitata mass rearing

Ceratitis capitata adults came from the permanent mass rearing maintained at the Department of Agriculture, Food and Environment (DAFE) of the University of Pisa. Adults were kept in 75 × 75 × 115 cm tents (BugDorm-2400 Insect Rearing Tent) provided with water and the solid diet made of sucrose and yeast extract 4:1 *w/w ad libitum* under laboratory conditions (23 ± 2 °C, 60–65% RH, natural photoperiod). Five-12-day old adults were used in the toxicity bioassays. Females laid eggs through the knitted mesh (hole size 650 μm) of the tents, and these fell into water-filled polyvinyl chloride trays (30 × 15 × 4 cm). Three times a week, eggs were sifted and distributed on a medium made of organic wheat bran (28%), sucrose (17%), yeast extract (11%), citric

acid (1%), and sodium benzoate (1%) mixed with water (42%), into which eggs and then larvae developed. When wandering, meaning when they voluntarily left the bran substrate after about ten days from egg hatching, we used part of the third-instar larvae in the toxicity bioassays and let part of them pupate and then emerge as adults in about six days to continue the cycle again or to be used in the toxicity bioassays.

2.4. Fumigation toxicity trials

The four EOs were tested for their toxicity by fumigation on the two Diptera pests. Groups of ten adults of undetermined sex or wandering larvae were put in cylindrical glass jars (volume 330 mL) sealed with screw lids (diameter 6.5 cm). Filter paper was used to cover the inner bottom of the jars to absorb excess humidity. Each jar contained a cotton roll soaked with water and sucrose (placed on a 7 mL weighing boat) as a nourishment for adult flies or 3 g of organic wheat bran as a support for wandering larvae, as they do not eat anymore. Under the lids, we placed a square of filter paper, poured with, in turn, at least five different amounts of one of the tested EOs to obtain a scale of mortality rates. We set a maximum limit of 350 μL (corresponding to 1060.50 μL EO L^{-1} air) to avoid the EO from dripping inside the jar. The exact quantities of EOs employed for *Ca. vomitoria* and *Ce. capitata* adults and wandering larvae are reported in Table 1. For amounts below 2.0 μL, we poured on the filter paper 100 μL of ethanol (EtOH) solutions of the EOs at the needed concentrations and allowed the solvent to evaporate under a fume hood before the beginning of the test. To avoid direct contact of the specimens with the EO, we secured a cotton gauze between the lid and the jar using a rubber band. For each EO and concentration tested, we replicated the test four times ($n = 4$), as well as for the control samples (consisting in the application of 100 μL of EtOH on the squares of filter paper). The actual fumigation lasted 24 h, then we moved the groups of insects into clean polypropylene cages with a netted lid for ventilation and provided with the appropriate feed (water and sucrose for adults) or support (bran for larvae). Mortality was checked after 24 more hours (*i.e.*, 48 h after the beginning of the trial).

2.5. Direct contact toxicity trials

The four EOs were tested for their toxicity by direct contact on the two Diptera pests. Groups of ten adults of undetermined sex or wandering larvae were individually topically treated by applying a drop of, in turn, at least four different concentrations (*v/v*) of the EOs in EtOH through a hand micro-applicator (Burkard Scientific Ltd., Uxbridge, UK). To ease the drop administration, *Ca. vomitoria* and *Ce. capitata* adults were put in a Falcon tube with a netted cap and anesthetized at −18 °C for a maximum of 180 and 60 s, respectively. The concentrations were selected to obtain a scale of mortality rates. We set a maximum limit of 2.0 μL of pure (100%) EO for *Ca. vomitoria* specimens and 1.0 μL of pure (100%) EO for *Ce. capitata* specimens according to the different dimensions of the body surface. Adults were treated on the notum and wandering larvae on the urotergites. The exact concentrations (and corresponding doses) employed for *Ca. vomitoria* and *Ce. capitata* adults and wandering larvae are reported in Table 1. Each group of insects was kept in a clean polypropylene cage with a netted lid for ventilation and provided with the appropriate feed (water and sucrose for adults) or support (bran for larvae). For each EO and dose tested, we replicated the test four times ($n = 4$), as well as for the control samples (consisting in the application of 1.0 or 2.0 μL of EtOH based on the species). Mortality was checked 48 h after the beginning of the trial.

2.6. Data analyses

As mortality in the control samples was always below 5% consistently for both the Diptera species, developmental stage, and modality of administration, we did not need to correct the values. The median lethal concentrations (LC_{50}), concentrations lethal for 95% of specimens (LC_{95}), median lethal doses (LD_{50}), and doses lethal for 95% of specimens (LD_{95}) were estimated by the probit model regression using the JMP Pro Software v 17.2 (SAS Institute Inc., Cary, NC). For all the LC_{50}, LC_{95}, LD_{50}, and LD_{95} values, we also calculated their respective 95% confidence intervals (95% CIs).

Table 1
Amounts of essential oils (EOs) used in the fumigation toxicity trials and concentrations (*v/v*) of the EOs in ethanol used in the direct contact toxicity trials involving *Calliphora vomitoria* and *Ceratitis capitata* adults and wandering larvae.

Fumigation				
Plant species EO	***Ca. vomitoria* adults**	***Ca. vomitoria* wandering larvae**	***Ce. capitata* adults**	***Ce. capitata* wandering larvae**
A. sativum	From 0.1–1.0 μL (from 0.30 to 3.03 μL EO L^{-1} air)	From 3.0–50 μL (from 9.09 to 151.50 μL EO L^{-1} air)	From 0.005–2.0 μL (from 0.015 to 6.06 μL EO L^{-1} air)	From 5.0–100 μL (from 15.15 to 303 μL EO L^{-1} air)
C. verum	From 20–250 μL (from 60.6 to 757.57 μL EO L^{-1} air)	From 50–350 μL (from 151.50 to 1060.50 μL EO L^{-1} air)	From 1.0–15 μL (from 3.03 to 4.45 μL EO L^{-1} air)	From 50–350 μL (from 151.50 to 1060.50 μL EO L^{-1} air)
O. vulgare	From 2.0–25 μL (from 6.06 to 75.75 μL EO L^{-1} air)	From 50–350 μL (from 151.50 to 1060.50 μL EO L^{-1} air)	From 1.0–6.0 μL (from 3.03 to 18.18 μL EO L^{-1} air)	From 50–350 μL (from 151.50 to 1060.50 μL EO L^{-1} air)
R. graveolens	From 1.0–8.0 μL (from 3.03 to 24.24 μL EO L^{-1} air)	From 50–350 μL (from 151.50 to 1060.50 μL EO L^{-1} air)	From 0.05–3.0 μL (from 0.15 to 9.09 μL EO L^{-1} air)	From 50–350 μL (from 151.50 to 1060.50 μL EO L^{-1} air)
Contact				
Plant species EO	***Ca. vomitoria* adults**	***Ca. vomitoria* wandering larvae**	***Ce. capitata* adults**	***Ce. capitata* wandering larvae**
A. sativum	From 0.05% to 2.0% (from 0.001 to 0.04 μL EO specimen^{-1})	From 25% to 100% (from 0.5 to 2.0 μL EO specimen^{-1})	From 0.25% to 1.5% (from 0.0025 to 0.015 μL EO specimen^{-1})	From 25% to 100% (from 0.25 to 1.0 μL EO specimen^{-1})
C. verum	From 1.0% to 9.0% (from 0.02 to 0.18 μL EO specimen^{-1})	From 25% to 100% (from 0.5 to 2.0 μL EO specimen^{-1})	From 0.5% to 4.0% (from 0.005 to 0.04 μL EO specimen^{-1})	From 25% to 100% (from 0.25 to 1.0 μL EO specimen^{-1})
O. vulgare	From 0.5% to 10.0% (from 0.01 to 0.2 μL EO specimen^{-1})	From 50% to 100% (from 1.0 to 2.0 μL EO specimen^{-1})	From 0.5% to 5.0% (from 0.005 to 0.05 μL EO specimen^{-1})	From 25% to 100% (from 0.25 to 1.0 μL EO specimen^{-1})
R. graveolens	From 2.0% to 12% (from 0.04 to 0.24 μL EO specimen^{-1})	From 25% to 100% (from 0.5 to 2.0 μL EO specimen^{-1})	From 0.5% to 4.0% (from 0.005 to 0.04 μL EO specimen^{-1})	From 25% to 100% (from 0.25 to 1.0 μL EO specimen^{-1})

Plant species essential oil = *Allium sativum*, *Cinnamomum verum*, *Origanum vulgare*, *Ruta graveolens.* Concentrations and doses (in brackets) are given as μL EO L^{-1} air for fumigation (considering the jar volume of 330 mL) and μL EO specimen^{-1} for contact (based on the drop volume of 2.0 μL for *Ca. vomitoria* and 1.0 μL for *Ce. capitata*), respectively.

To compare the LC_{50} and LD_{50} values, we derived the relative toxicity (RT) as the ratio between the LC_{50} or LD_{50} values for each EO and the LC_{50} or LD_{50}, respectively for our most effective EO, *i.e.*, the *A. sativum* one. The RT values in tables represent how many times more toxic the *A. sativum* EO is relative to the other EO considered. We also calculated the 95% confidence intervals (95% CIs) of the RT values.

The susceptibility ratios (SR) were derived as the ratio between the LC_{50} or LD_{50} values for wandering larvae and/or adults of the same species or the other species (*i.e.*, *Ca. vomitoria* adults *vs*. *Ce. capitata* adults; *Ca. vomitoria* wandering larvae *vs*. *Ce. capitata* wandering larvae; *Ca. vomitoria* wandering larvae *vs*. *Ca. vomitoria* adults; *Ce. capitata* wandering larvae *vs*. *Ce. capitata* adults). We also calculated the 95% confidence intervals (95% CIs) of the SR values.

3. Results

3.1. Essential oils compositions

The complete chemical composition of the four selected EOs is reported in Table 2.

Eighteen compounds were identified in the *A. sativum* EO, with a prevalence of diallyl trisulfide (41.8%), diallyl disulfide (18.1%), and diallyl tetrasulfide (16.4%). Indeed, 93.0% of the composition was represented by sulfur derivatives. The *C. verum* EO encompassed 30 constituents, with (*E*)-cinnamaldehyde (65.1%) being the prevailing one. Overall, the phenylpropanoid class accounted for 78.5% of the composition. The *O. vulgare* EO exhibited a carvacrol chemotype, containing 70.2% of this monoterpenoid phenol among a total of 24 identified components. Oxygenated monoterpenes represented 82.2% of its total composition. The *R. graveolens* EO contained 19 detected compounds, with a notable 86.2% of 2-undecanone. This and other non-terpene derivatives accounted for 92.0% of the composition.

3.2. Fumigation and contact toxicity on Calliphora vomitoria adults and wandering larvae

The results of the toxicity trials performed on *Ca. vomitoria* adult flies are reported in Table 3. The *A. sativum* EO was the most effective through both modalities of administration, rendering an LC_{50} by fumigation of 1.702 μL EO L^{-1} air and an LD_{50} by contact of 0.006 μL EO $specimen^{-1}$. The other EOs showed lower bioactivity (RT values > 1.0) on the pest also according to the trial type (by a factor from RT (95% CIs) = 6.49 (5.79 – 7.27) to 240.78 (216.63 – 267.61) for fumigation and from RT = 5.83 (4.00 – 8.50) to 27.50 (20.63 – 36.67) for contact) and composition. For instance, the *O. vulgare* EO exerted greater toxicity by contact than fumigation, while it was the opposite for the *R. graveolens* one. As for the *C. verum* EO, it was poorly active by fumigation (LC_{50} = 409.800 μL EO L^{-1} air, meaning 240.78-fold less effective than the *A. sativum* EO).

The results of the toxicity trials performed on *Ca. vomitoria* wandering larvae are reported in Table 4. The only EO able to generate more than 95% mortality through both modalities of administration was the *A. sativum* one, with an LC_{50} of 26.627 μL EO L^{-1} air and an LD_{50} of 0.937 μL EO $specimen^{-1}$. The *O. vulgare* EO was active only by contact but less than the *A. sativum* EO (by a factor of 1.36), as having an LD_{50} of 1.277 μL EO $specimen^{-1}$. The other EOs, at the highest concentration tested by fumigation according to the limit we set, namely 1060.50 μL EO L^{-1} air, were not toxic at all, meaning that they rendered 0% mortality. At the highest dose tested by contact according to the limit we set, namely 2.0 μL EO $specimen^{-1}$, the *R. graveolens* EO caused 0% mortality and the *C. verum* one 20.0 ± 8.16% (mean mortality ± standard deviation - SD, $n = 4$).

The LD_{95} for the *O. vulgare* EO was predicted by the probit model regression as 2.011 μL EO $specimen^{-1}$. At the maximum tested dose of 2.0 μL EO $specimen^{-1}$, in fact, we obtained 90.0 ± 7.08% (mean mortality ± SD, $n = 4$) mortality. This estimate, therefore, suggests a partial unsuitability of this EO for a satisfying control of *Ca. vomitoria* at the larval stage through contact.

3.3. Fumigation and contact toxicity on Ceratitis capitata adults and wandering larvae

The results of the toxicity trials performed on *Ce. capitata* adult flies are reported in Table 5. Even in this case, the *A. sativum* EO was the most effective through both modalities of administration, with an LC_{50} by fumigation of 1.238 μL EO L^{-1} air and an LD_{50} by contact of 0.007 μL EO $specimen^{-1}$. The other EOs showed lower bioactivity (RT values > 1.0) on the pest and different outcomes according to the trial type (by a factor from RT (95% CIs) = 1.74 (1.24 – 2.42) to 12.59 (9.76 – 16.24) for fumigation and from RT = 2.00 (1.58 – 2.53) to 2.86 (2.37 – 3.45) for contact) and composition. The *C. verum* EO, conversely to what was observed on *Ca. vomitoria* adults, showed decent potential as a fumigant (LC_{50} = 15.587 μL EO L^{-1} air *vs*. 409.800 μL EO L^{-1} air for *Ca. vomitoria* adults), even if it was the least effective (12.59-fold less than the *A. sativum* EO). Furthermore, it was the second-best performing by contact (LD_{50} = 0.014 μL EO $specimen^{-1}$), similarly to the *R. graveolens* EO (0.018 μL EO $specimen^{-1}$).

The results of the toxicity trials performed on *Ce. capitata* wandering larvae are reported in Table 6. The only EO able to generate more than 50% mortality through both modalities of administration was the *A. sativum* one, with an LC_{50} of 89.653 μL EO L^{-1} air and an LD_{50} of 0.751 μL EO $specimen^{-1}$. The other EOs, at the highest concentration tested by fumigation according to the limit we set, namely 1060.50 μL EO L^{-1} air, were less effective or not toxic at all. In detail, the *O. vulgare* and *R. graveolens* EOs rendered 0% mortality and the C. *verum* one 10 ± 3.54% (mean mortality ± SE, $n = 4$). At the highest dose tested by contact according to the limit we set, namely 1.0 μL EO $specimen^{-1}$, the *C. verum* and *R. graveolens* EOs caused 0% mortality and the *O. vulgare* one 4.34 ± 2.17% (mean mortality ± SD, $n = 4$).

The LD_{95} for the *A. sativum* EO was predicted by the probit model regression as 1.364 μL EO $specimen^{-1}$. At the maximum tested dose of 1.0 μL EO $specimen^{-1}$, in fact, we obtained 70.0 ± 15.82% (mean mortality ± SD, $n = 4$) mortality. This estimate, therefore, suggests the unsuitability of this EO for a satisfying control of *Ce. capitata* at the larval stage through contact.

3.4. Differences in susceptibility

Adults of the two Diptera pests showed different susceptibility according to the EO composition and administration modality, as shown in Table 7. In detail, *Ca. vomitoria* adults were always less susceptible (SR values > 1.0) by fumigation [SR (95% CIs) = 1.37 (1.08 – 1.74) to 26.29 (22.84 – 30.26)] and by contact [SR = 1.75 (1.32 – 2.32) to 9.17 (8.05 – 10.44)] with the *C. verum*, *O. vulgare,* and *R. graveolens* EOs. Only with the *A. sativum* EO applied by contact, *Ca. vomitoria* adults seemed slightly more susceptible (SR < 1.0, namely 0.86) than *Ce. capitata* ones, although this difference is not statistically significant due to the overlapping CIs (0.63 – 1.179).

Regarding the values for the *A. sativum* EO efficacy on wandering larvae, *Ce. capitata* is 3.4-fold less susceptible than *Ca. vomitoria* by fumigation [SR = 0.30 (0.21 – 0.42)], while it is the opposite by contact, being *Ca. vomitoria* slightly less susceptible [SR = 1.25 (1.01 – 1.54)] in this case.

Interestingly, when comparing the LC_{50} and LD_{50} values obtained for adults and wandering larvae belonging to the same species, we can always observe a pronounced lower susceptibility of the latter. For instance, while the *C. verum*, *O. vulgare*, and *R. graveolens* EOs were able to kill even more than 95% of adults by both fumigation and direct contact, the same bioactivity was not observed on wandering larvae. Focusing on the most toxic EO among the four here selected, namely the *A. sativum* one, we obtained elevated SR values both for *Ca. vomitoria* and *Ce. capitata* by fumigation and, especially, direct contact (*Ca.*

Table 2
Chemical composition (compounds > 0.1%) of the *Allium sativum*, *Cinnamomum verum*, *Origanum vulgare*, and *Ruta graveolens* essential oils.

Compounds	l.r.i.	Relative abundance (%) ± SD			
		A. sativum	***C. verum***	***O. vulgare***	***R. graveolens***
(*E*)-allyl (prop-1-en-1-yl) sulfane	891	5.1 ± 0.93	-	-	-
methyl allyl disulfide	920	1.6 ± 0.22	-	-	-
α-thujene	931	-	-	0.1 ± 0.03	-
(*E*)-methylpropenyl disulfide	940	0.1 ± 0.01	-	-	-
α-pinene	941	-	0.3 ± 0.07	0.4 ± 0.08	0.6 ± 0.10
camphene	955	-	0.1 ± 0.09	0.1 ± 0.02	0.4 ± 0.07
benzaldehyde	959	-	0.2 ± 0.04	-	-
dimethyl trisulfide	975	0.5 ± 0.04	-	-	-
sabinene	976	-	-	-	0.3 ± 0.03
β-pinene	982	-	0.1 ± 0.03	0.6 ± 0.11	0.2 ± 0.02
3-octanone	987	-	-	0.1 ± 0.00	-
myrcene	993	-	-	0.4 ± 0.05	-
α-phellandrene	1005	-	0.7 ± 0.13	-	-
α-terpinene	1018	-	0.4 ± 0.06	0.5 ± 0.06	-
p-cymene	1027	-	1.3 ± 0.16	6.2 ± 0.57	0.4 ± 0.04
β-phellandrene	1031	-	1.7 ± 0.23	-	-
limonene	1032	-	-	0.5 ± 0.06	0.8 ± 0.11
1,8-cineole	1034	-	0.1 ± 0.07	0.9 ± 0.06	1.3 ± 0.14
γ-terpinene	1062	-	-	3.7 ± 0.39	-
diallyl disulfide	1082	18.1 ± 1.10	-	-	-
terpinolene	1088	-	0.1 ± 0.01	-	-
2-nonanone	1094	-	-	-	2.7 ± 0.16
linalool	1101	-	2.7 ± 0.16	2.9 ± 0.13	0.3 ± 0.01
(*E*)-1-allyl-2-(prop-1-en-1-yl) disulfane	1103	0.5 ± 0.01	-	-	-
nonanal	1104	-	-	-	0.1 ± 0.01
(*Z*)-1-allyl-2-(prop-1-en-1-yl) disulfane	1107	0.4 ± 0.01	-	-	-
camphor	1143	-	-	0.8 ± 0.01	0.3 ± 0.02
4-methyl-1,2,3-trithiolane	1154	0.7 ± 0.12	-	-	-
hydrocinnamaldehyde	1161	-	0.4 ± 0.01	-	-
borneol	1165	-	-	1.1 ± 0.03	-
4-terpineol	1178	-	0.3 ± 0.01	0.6 ± 0.01	-
α-terpineol	1189	-	0.5 ± 0.01	0.7 ± 0.01	-
2-decanone	1193	-	-	-	1.5 ± 0.04
2-vinyl-4H-1,3-dithiine	1206	0.5 ± 0.03	-	-	-
dimethyl tetrasulfide	1210	0.3 ± 0.05	-	-	-
(*Z*)-cinnamaldehyde	1217	-	0.5 ± 0.01	-	-
methyl carvacrol	1239	-	-	0.3 ± 0.02	-
(*E*)-cinnamaldehyde	1268	-	65.1 ± 0.83	-	-
thymol	1292	-	-	4.8 ± 0.03	-
2-undecanone	1294	-	-	-	86.2 ± 0.57
diallyl trisulfide	1297	41.8 ± 0.16	-	-	-
carvacrol	1298	-	-	70.2 ± 1.6	-
(*E*)-cinnamyl alcohol	1304	-	0.2 ± 0.00	-	-
2-undecanol	1308	-	-	-	0.2 ± 0.03
eugenol	1358	-	6.1 ± 0.05	0.2 ± 0.00	-
5-methyl-1,2,3,4-tetrathiane	1364	0.7 ± 0.08	-	-	-
hydroxycinnamyl acetate	1367	-	0.2 ± 0.00	-	-
α-copaene	1376	-	1.0 ± 0.06	-	-
β-caryophyllene	1420	-	7.2 ± 0.04	3.5 ± 0.02	0.9 ± 0.01
2-undecanol acetate	1433	-	-	-	0.7 ± 0.01
(*E*)-cinnamyl acetate	1444	-	5.4 ± 0.06	-	-
α-humulene	1456	-	1.5 ± 0.03	0.6 ± 0.01	-
2-tridecanone	1496	-	-	-	0.5 ± 0.02
β-bisabolene	1509	-	-	0.2 ± 0.02	-
eugenol acetate	1528	-	0.5 ± 0.01	-	-
diallyl tetrasulfide	1540	16.4 ± 1.17	-	-	-
palustrol	1568	-	0.1 ± 0.07	-	-
caryophyllene oxide	1581	-	1.0 ± 0.08	0.8 ± 0.01	1.8 ± 0.07
1-(1-(prop-1-en-1ylthio)propyl)-2-propyl disulfane	1592	0.3 ± 0.06	-	-	-
6-methyl-4,5,8-trithia-1,10-undecadiene	1597	0.2 ± 0.01	-	-	-
6-ethyl-4,5,7-trithia-2,8-decadiene	1603	0.8 ± 0.09	-	-	-
humulene epoxide II	1608	-	0.1 ± 0.00	-	-
trans-3,6-diethyl-1,2,4,5-tetrathiane	1613	0.3 ± 0.01	-	-	-
tetradecanal	1614	-	0.5 ± 0.01	-	-
14-hydroxy-9-*epi*-(*E*)-caryophyllene	1664	-	-	-	0.2 ± 0.01
benzyl benzoate	1764	-	1.5 ± 0.04	-	-
Monoterpene hydrocarbons		1.6 ± 0.22	4.6 ± 0.78	12.5 ± 1.36	2.7 ± 0.36
Oxygenated monoterpenes		-	3.5 ± 0.26	82.2 ± 1.31	1.9 ± 0.18
Sesquiterpene hydrocarbons		-	9.9 ± 0.06	4.3 ± 0.06	0.9 ± 0.01
Oxygenated sesquiterpenes		-	1.2 ± 0.15	0.8 ± 0.01	2.0 ± 0.08
Phenylpropanoids		-	78.5 ± 0.92	0.2 ± 0.00	-
Sulfur compounds		93.0 ± 0.37	-	-	-
Non-terpene derivatives		-	2.3 ± 0.01	0.1 ± 0.0	92.0 ± 0.43
Total identified (%)		94.6 ± 0.59	100 ± 0.01	100 ± 0.01	99.5 ± 0.01

l.r.i. = linear retention index on an HP-5MS capillary column; SD = standard deviation ($n = 3$); - = not detected.

Table 3
Toxicity by fumigation and contact given by essential oils (EOs) on *Calliphora vomitoria* adults (48 h from the beginning of the trial) and relative toxicity of the EOs compared to the *Allium sativum* one for LC_{50} and LD_{50} values.

Fumigation							
Plant species EO	**n**	**LC_{50} (95% CIs)**	**LC_{95} (95% CIs)**	**Slope ± SE**	**χ^2 (df)**	***p***	**RT (95% CIs)**
A. sativum	32	1.702 (1.589 – 1.829)	2.774 (2.550 – 3.092)	1.534 ± 0.150	29.437 (30)	0.495	
C. verum	56	409.800 (379.412 – 444.217)	812.002 (742.116 – 904.964)	0.004 ± 0.001	55.077 (54)	0.434	240.78 (216.63 – 267.61)
O. vulgare	40	35.779 (32.789 – 38.921)	70.704 (64.370 – 79.558)	0.047 ± 0.004	44.126 (38)	0.228	21.02 (18.82 – 23.49)
R. graveolens	32	11.045 (10.102 – 12.063)	20.744 (18.741 – 23.692)	0.170 ± 0.018	35.340 (30)	0.230	6.49 (5.79 – 7.27)
Contact							
Plant species EO	**n**	**LD_{50} (95% CIs)**	**LD_{95} (95% CIs)**	**Slope ± SE**	**χ^2 (df)**	***p***	**RT (95% CIs)**
A. sativum	28	0.006 (0.004 – 0.007)	0.019 (0.016 – 0.023)	124.313 ± 15.965	12.753 (26)	0.986	
C. verum	24	0.104 (0.091 – 0.117)	0.226 (0.199 – 0.271)	13.430 ± 1.758	26.365 (22)	0.236	17.33 (12.75 – 23.56)
O. vulgare	24	0.035 (0.026 – 0.043)	0.104 (0.087 – 0.133)	23.907 ± 3.650	14.054 (22)	0.900	5.83 (4.00 – 8.50)
R. graveolens	24	0.165 (0.154 – 0.176)	0.255 (0.235 – 0.286)	18.137 ± 2.076	22.186 (22)	0.449	27.50 (20.63 – 36.67)

Plant species essential oil = *Allium sativum*, *Cinnamomum verum*, *Origanum vulgare*, *Ruta graveolens*; n = number of observations; LC_{50} or LD_{50} = concentration or dose that kills 50% of the specimens subjected to it; LC_{95} or LD_{95} = concentration or dose that kills 95% of the specimens subjected to it; CIs = confidence intervals; SE = standard error; χ^2 = chi-square; df = degrees of freedom; p = Pearson goodness-of-fit test; RT = relative toxicity compared to the *A. sativum* EO for LC_{50} and LD_{50} values. Data are given as μL EO L^{-1} air for fumigation and μL EO $specimen^{-1}$ for contact.

Table 4
Toxicity by fumigation and contact given by essential oils (EOs) on *Calliphora vomitoria* wandering larvae (48 h from the beginning of the trial) and relative toxicity of the *Origanum vulgare* EO compared to the *Allium sativum* one for the LD_{50} value.

Fumigation							
Plant species EO	**n**	**LC_{50} (95% CIs)**	**LC_{95} (95% CIs)**	**Slope ± SE**	**χ^2 (df)**	***p***	
A. sativum	24	26.627 (19.153 – 34.220)	88.758 (72.481 – 118.866)	0.026 ± 0.004	33.438 (22)	0.056	
Contact							
Plant species EO	**n**	**LD_{50} (95% CIs)**	**LD_{95} (95% CIs)**	**Slope ± SE**	**χ^2 (df)**	***p***	**RT (95% CIs)**
A. sativum	16	0.937 (0.765 – 1.079)	1.96 (1.729 – 2.365)	1.600 ± 0.240	9.170 (14)	0.820	
O. vulgare	20	1.277 (1.176 – 1.363)	2.011 (1.839 – 2.319)	2.240 ± 0.350	16.251 (18)	0.575	1.36 (1.13 – 1.64)

Plant species essential oil = *Allium sativum*, *Cinnamomum verum*, *Origanum vulgare*, *Ruta graveolens*; n = number of observations; LC_{50} or LD_{50} = concentration or dose that kills 50% of the specimens subjected to it; LC_{95} or LD_{95} = concentration or dose that kills 95% of the specimens subjected to it; CIs = confidence intervals; SE = standard error; χ^2 = chi-square; df = degrees of freedom; p = Pearson goodness-of-fit test; RT = relative toxicity compared to the *A. sativum* EO for the LD_{50} value. Data are given as μL EO L^{-1} air for fumigation and μL EO $specimen^{-1}$ for contact.

vomitoria: SR = 15.64 (11.61 – 21.09) by fumigation and 156.17 (112.45 – 216.88) by contact; *Ce. capitata*: SR = 72.42 (53.90 – 97.31) by fumigation and 107.29 (89.09 – 129.19) by contact), thus highlighting a marked ontogenic difference (*i.e.*, wandering larvae are less susceptible than adults).

4. Discussion

As expected from the literature analysis performed, the compositions of the four EOs selected for our trials are in line with others previously reported and reflect the prevalence of sulfur derivatives, phenylpropanoids, oxygenated monoterpenes, or non-terpene derivatives as intended. Garlic EOs from bulbs are typically composed of over 90% of sulfur compounds, including diallyl sulfide, disulfide, trisulfide, and tetrasulfide (Palermo et al., 2021). The *A. sativum* EO here tested contains a total of 93.0% sulfur compounds, encompassing diallyl trisulfide (41.8%), diallyl disulfide (18.1%), and diallyl tetrasulfide (16.4%). Cinnamon bark EOs are usually characterized by phenylpropanoids, with (*E*)-cinnamaldehyde as the major component (40–80%) and eugenol in lower amounts, thus highlighting the prevalence of such chemical class in their composition (Aungtikun and Soonwera, 2021). Phenylpropanoids account for 78.5% of our *C. verum* EO, with a marked prevalence of (*E*)-cinnamaldehyde (65.1%), followed by eugenol (6.1%). Based on the most abundant oxygenated monoterpene detected, the thymol and carvacrol chemotypes can be identified in oregano EOs; further chemotypes are then added by referring to the second most represented component (Raal et al., 2024). Our *O. vulgare* EO, having 70.2% of carvacrol, can be classified accordingly. Rue EOs from leaves/aerial parts were previously characterized by other authors, and as for our *R. graveolens* EO, they highlighted a dominance of 2-undecanone (86.2% in our EO) and/or 2-nonanone (2.7% in this case), two ketones categorized among the non-terpene derivatives (Lee et al., 2023).

Table 5
Toxicity by fumigation and contact given by essential oils (EOs) on *Ceratitis capitata* adults (48 h from the beginning of the trial).

Fumigation							
Plant species EO	**n**	**LC_{50} (95% CIs)**	**LC_{95} (95% CIs)**	**Slope ± SE**	**χ^2 (df)**	***p***	**RT (95% CIs)**
A. sativum	32	1.238 (0.971 – 1.527)	3.979 (3.373 – 4.923)	0.600 ± 0.071	31.834 (30)	0.375	
C. verum	28	15.587 (13.791 – 17.406)	31.002 (27.867 – 35.536)	0.107 ± 0.011	12.479 (26)	0.988	12.59 (9.76 – 16.24)
O. vulgare	24	10.033 (9.044 – 11.012)	18.466 (16.733 – 21.033)	0.195 ± 0.021	10.626 (22)	0.979	8.10 (6.33 – 10.37)
R. graveolens	28	2.150 (1.628 – 2.655)	6.699 (5.776 – 8.084)	0.361 ± 0.041	43.949 (26)	0.015	1.74 (1.24 – 2.42)
Contact							
Plant species EO	**n**	**LD_{50} (95% CIs)**	**LD_{95} (95% CIs)**	**Slope ± SE**	**χ^2 (df)**	***p***	**RT (95% CIs)**
A. sativum	20	0.007 (0.006 – 0.008)	0.014 (0.012 – 0.017)	212.214 ± 29.441	15.181 (18)	0.649	
C. verum	28	0.014 (0.011 – 0.016)	0.034 (0.030 – 0.040)	81.367 ± 9.846	21.336 (26)	0.724	2.00 (1.58 – 2.53)
O. vulgare	24	0.020 (0.018 – 0.023)	0.041 (0.037 – 0.048)	78.161 ± 8.450	14.439 (22)	0.885	2.86 (2.37 – 3.45)
R. graveolens	24	0.018 (0.016 – 0.020)	0.036 (0.032 – 0.041)	90.625 ± 10.239	17.878 (22)	0.713	2.57 (2.14 – 3.08)

Plant species essential oil = *Allium sativum*, *Cinnamomum verum*, *Origanum vulgare*, *Ruta graveolens*; n = number of observations; LC_{50} or LD_{50} = concentration or dose that kills 50% of the specimens subjected to it; LC_{95} or LD_{95} = concentration or dose that kills 95% of the specimens subjected to it; CIs = confidence intervals; SE = standard error; χ^2 = chi-square; df = degrees of freedom; *p* = Pearson goodness-of-fit test; RT = relative toxicity compared to the *A. sativum* EO for LC_{50} and LD_{50} values. Data are given as μL EO L^{-1} air for fumigation and μL EO $specimen^{-1}$ for contact.

Table 6
Toxicity by fumigation and contact given by *Allium sativum* essential oil on *Ceratitis capitata* wandering larvae (48 h from the beginning of the trial).

Fumigation					
n	**LC_{50} (95% CIs)**	**LC_{95} (95% CIs)**	**Slope ± SE**	**χ^2 (df)**	***p***
24	89.653 (74.178 – 108.427)	223.519 (188.073 – 282.628)	0.012 ± 0.002	17.782 (22)	0.719
Contact					
n	**LD_{50} (95% CIs)**	**LD_{95} (95% CIs)**	**Slope ± SE**	**χ^2 (df)**	***p***
16	0.751 (0.671 – 0.849)	1.364 (1.184 – 1.697)	2.684 ± 0.433	11.136 (14)	0.675

n = number of observations; LC_{50} or LD_{50} = concentration or dose that kills 50% of the specimens subjected to it; LC_{95} or LD_{95} = concentration or dose that kills 95% of the specimens subjected to it; CIs = confidence intervals; SE = standard error; χ^2 = Chi-Square; df = degrees of freedom; *p* = Pearson goodness-of-fit test. Data are given as μL EO L^{-1} air for fumigation and μL EO $specimen^{-1}$ for contact.

Table 7
Susceptibility ratio (SR) between *Calliphora vomitoria* and *Ceratitis capitata* LC_{50} or LD_{50} values on adults given by essential oils (EOs).

Plant species EO	**SR by fumigation (95% CIs)**	**SR by contact (95% CIs)**
A. sativum	1.37 (1.08 – 1.74)	0.86 (0.63 – 1.17)
C. verum	26.29 (22.84 – 30.26)	7.43 (5.93 – 9.31)
O. vulgare	3.57 (3.13 – 4.06)	1.75 (1.32 – 2.32)
R. graveolens	5.14 (3.96 – 6.66)	9.17 (8.05 – 10.44)

Plant species essential oil = *Allium sativum*, *Cinnamomum verum*, *Origanum vulgare*, *Ruta graveolens*; LC_{50} or LD_{50} = concentration or dose that kills 50% of the specimens subjected to it; SR = susceptibility ratio; CIs = confidence intervals. The value < 1.0 indicates that *Ca. vomitoria* is more susceptible, although the trend is not statistically significant due to the overlapping CIs; values > 1.0 indicate that *Ce. capitata* is more susceptible.

Exhaustive reviews going through the numerous pharmaceutical, culinary, agricultural, and insecticidal properties of garlic, cinnamon, oregano, and rue are already available. Here, we limit our discussion to the different susceptibility based on the target pest and ontogeny and the lethal effects of sulfur derivatives on insects.

For the first time in this paper, we highlighted lower susceptibility of *Ca. vomitoria* to the selected EOs compared to *Ce. capitata*. The necessity to use higher concentrations and doses of EOs to manage *Ca. vomitoria* than *Ce. capitata* (meaning overall greater LC_{50} or LC_{95} and LD_{50} or LD_{95} values) might be related to the more than three-fold bigger size of the former compared to the latter. *Calliphora vomitoria* adults are 10–14 mm long and wandering larvae about 16–18 mm (Farina et al., 2026). In *Ce. capitata*, adults measure 3.5–5 mm in length and wandering larvae 6.8–8.2 mm (OEPP/EPPO, 2011). However, in couples of species closely related to each other, for instance *Sitophilus oryzae* (Linnaeus) with *Sitophilus zeamais* Motschulsky (Coleoptera Dryophthoridae) and *Tribolium castaneum* (Herbst) with *Tribolium confusum* J. du Val (Coleoptera Tenebrionidae), the toxic effect of some tested EOs was drastically different, despite the pests being equivalent in dimensions (Abbad et al., 2014; Song et al., 2016). Therefore, species-specific susceptibility to EOs must come into play regardless of body size.

Following a size/weight-related principle, we might assume different susceptibility between larval instars and imagoes of the same species. This phenomenon was verified in the mosquito *Anopheles arabiensis* Patton (Diptera Culicidae) when exposed to five EO constituents (*i.e.*, *cis*-nerolidol, *trans*-nerolidol, (−)-α-bisabolol, farnesol, and methyl-eugenol), all exhibiting potent larvicidal activity on third instars (LC_{50} = 0.05–2.37 μM) but only moderate lethality against adults (LC_{50} = 222.29–323.69 μM) (Rants'o et al., 2023). Likewise, first instar larvae of *Microtheca ochroloma* Stål (Coleoptera Chrysomelidae) were about twice as susceptible to pyrethrum and 20-fold to Spinosad as their adults (Balusu and Fadamiro, 2013), and larvae of unspecified age of different Coleoptera pests of stored products were killed more easily than adult beetles by some chemical (Goto et al., 2004) and botanical (Plata-Rueda et al., 2017) agents.

Conversely, our results highlight, for the first time in these species, a markedly higher susceptibility of both Diptera adults compared to their relative wandering larvae by fumigation (SR = 15.64 for *Ca. vomitoria* and 72.42 for *Ce. capitata*) and, especially, direct contact (SR = 156.17

for *Ca. vomitoria* and 107.29 for *Ce. capitata*). We must notice that we worked with the last larval instar of both species (namely, the third), so such a discrepancy might be due to the age factor rather than to a simple matter of body size/weight. Indeed, topical applications of Spinosad on *T. castaneum* confirmed that young larvae (12-day-old) and adults have similar LD_{50} values 48 h post-treatment (1069 and 973 mg L^{-1}, respectively), whereas old larvae (22-day-old) are about 160-fold less susceptible (Yousefnezhad-Irani and Asghar, 2007). Still on *T. castaneum*, this phenomenon was evident when fumigating its different developmental stages with methyl allyl disulfide and diallyl trisulfide from a garlic EO (Huang et al., 2000). Mature larvae (third instars) of *Musca domestica* Linnaeus (Diptera Muscidae) had 205.52 and 180.14-fold lower susceptibility than adults to a pyrethroid and an organophosphate administered by contact, respectively (Zhu et al., 2002). Similarly, mature larvae (eighth instars) of *Alphitobius diaperinus* (Panzer) (Coleoptera Tenebrionidae) collected from infested broiler houses were always less susceptible than the corresponding adults to nine synthetic insecticides (*e.g.*, chlorinated hydrocarbons, organophosphates, pyrethroids) in residual and topical application tests (Steelman, 2008).

As indicated by Zhu et al. (2002), the pronounced tolerance of mature larvae might be associated with higher glutathione S-transferases (GST) activity. GST are part of a multifunctional protein family which regulates, by others, detoxification, oxidative stress response, and immune defense mechanisms in insects. Although the involvement of GST was not experimentally verified in our study, we believe that this hypothesis can appropriately explain the ontogenetic difference in susceptibility. Indeed, this phenomenon was confirmed in the housefly *M. domestica* (Subaharan et al., 2021) and Chinese citrus fruit fly *Bactrocera minax* (Enderlein) (Diptera Tephritidae) (Chen et al., 2012), two species close to our target pests. Analogously, seventh instar larvae of the model organism *Galleria mellonella* (Linnaeus) (Lepidoptera Pyralidae) (Çelik et al., 2024) and honeybee 8-day larvae (Choi et al., 2024) revealed higher GST base levels than their respective imagoes. The same occurrence, however, was not demonstrated in *T. castaneum* nor *Popillia japonica* Newman (Coleoptera Scarabaeidae) late larvae *vs*. adults (Adesanya et al., 2018; Deb and Kumar, 2020), but the results reported in the two studies suggest the possible involvement of cytochromes P450, another superfamily of detoxifying enzymes, in the different susceptibility degrees.

Despite all the EOs here tested exerting some level of toxicity on at least one of our Diptera target pests (considering both developmental stages and modalities of administration), the *A. sativum* one was the most effective, as shown by the side-by-side evaluation of their bioactivity through relative toxicity (RT) ratios. This superiority is likely due to its arsenal of sulfur compounds. Indeed, many of these were tested before and highlighted numerous toxic effects on the specimens subjected to them. It is no surprise that elemental sulfur is one of the oldest pesticides used as a metabolic disruptor and has been registered since the 1920s in the USA. In terms of acute toxicity, among the main sulfuric compounds found in an *Allium tuberosum* Rottler ex Spreng. (Amaryllidaceae) leaf EO and the EO itself, diallyl trisulfide rendered the lowest LD_{50} on *Apolygus lucorum* (Meyer-Dur) (Heteroptera Miridae) adults, namely 10.13 μg specimen^{-1} by direct contact (Shi et al., 2015). Similarly, diallyl trisulfide outperformed an *A. tuberosum* root EO and other major compounds when tested against *Aedes albopictus* (Skuse) (Diptera Culicidae) fourth instar larvae (LC_{50} = 4 μg mL^{-1}) (Liu et al., 2015). Diallyl trisulfide had also greater efficacy than a garlic EO when tested by direct contact on *Cacopsylla chinensis* (Yang & Li) (Hemiptera Psyllidae) overwintering adults (LC_{50} = 0.64 μg specimen^{-1}) (Zhao et al., 2013).

Diallyl sulfide and diallyl disulfide were more toxic to *Tuta absoluta* (Meyrick) (Lepidoptera Gelechiidae) second instar larvae when singularly administered through the leaf disc method than in binary mixtures at different ratios. The two compounds, at the estimated LC_{30} and LC_{50} concentrations, decreased the activity of digestive enzymes (*i.e.*, α-amylase, glucosidases, lipases, and proteases), acetylcholine esterase, alanine aminotransferase, and storage macromolecules (*i.e.*, triglycerides, glycogen, and proteins) (Talepour et al., 2021). Diallyl trisulfide significantly decreased the cuticular chitin content in *Sitotroga cerealella* (Olivier) (Lepidoptera Gelechiidae) adult moths when treated with a sub-lethal dose (Shah et al., 2021), as it downregulated the chitin synthase A gene (Shah et al., 2020). Furthermore, the compound reduced fecundity and fertility in females and overall altered the mating behavior (*i.e.*, lower mating frequency, calling percentage of females, copulation duration, and sex pheromone production) (Chang et al., 2019). On *S. zeamais* and *T. castaneum*, diallyl trisulfide suppressed egg hatching and the consequent larval and adult emergence and limited growth rate, food consumption, and food utilization (Huang et al., 2000).

Interestingly, exposure of *Callosobruchus maculatus* (Fabricius) (Coleoptera Chrysomelidae) to dimethyl disulfide from damaged leek plants increased the GST activity in its adults and mature larvae (fourth instars), suggesting the involvement of this enzyme also in the tolerance to this sulfurated allelochemical (Dugravot et al., 2004).

5. Conclusion

In conclusion, EOs possess the praised vast toxicity on insects, among other useful properties, but we cannot generalize, as their compositions are broadly varied. At the same time, an EO that is recommended against a specific pest does not guarantee satisfactory protection against all its developmental stages. Precise assessments are warranted to tailor control strategies that avoid, as much as possible, side effects on non-target organisms and, especially, particularly sensitive instars of such.

Garlic EO and its sulfurated phytoconstituents, notably diallyl trisulfide, are valid weapons against insect pests. Further assessments of toxic effects on *Ca. vomitoria* and *Ce. capitata* given by sulfur compounds-containing EOs or the singular molecules could add tools to the management prospects of the two pests of economic relevance at a practical level. To strengthen the mechanistic interpretation of the obtained results, further assessment spanning from *in vivo* and *in vitro* biochemical analyses to *in silico* molecular docking studies of the major sulfur derivatives against relevant insect molecular targets (*e.g.*, GST, cytochromes P450, acetylcholinesterase, gamma-aminobutyric acid, octopamine) could provide valuable insights into structure–activity relationships, supporting the observed stage-specific toxicity and detoxification-related hypotheses.

One of the limitations of garlic EO and sulfur derivatives is the pungent smell that might negatively impact human olfaction if proposed as a potential insecticide to protect, for instance, meat products (against blowflies) or fruit (against fruit flies). To reduce this inconvenience while increasing the EO water solubility, stability, and release over time, nano-formulation technologies could come to our help. Moreover, formulated EOs and singular compounds would allow reducing the concentrations or doses needed to achieve equivalent bioactivity on insect pests, with substantial economic and environmental benefits.

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Investigation. **Roberta Ascrizzi:** Writing – review & editing, Validation, Investigation. **Federica Semprucci:** Writing – review & editing, Funding acquisition. **Guido Flamini:** Writing – review & editing, Supervision, Resources. **Barbara Conti:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Funding acquisition, Conceptualization. **Loretta Guidi:** Writing – review & editing.

Declaration of Competing Interest

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

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Data availability

Data will be made available on request.

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