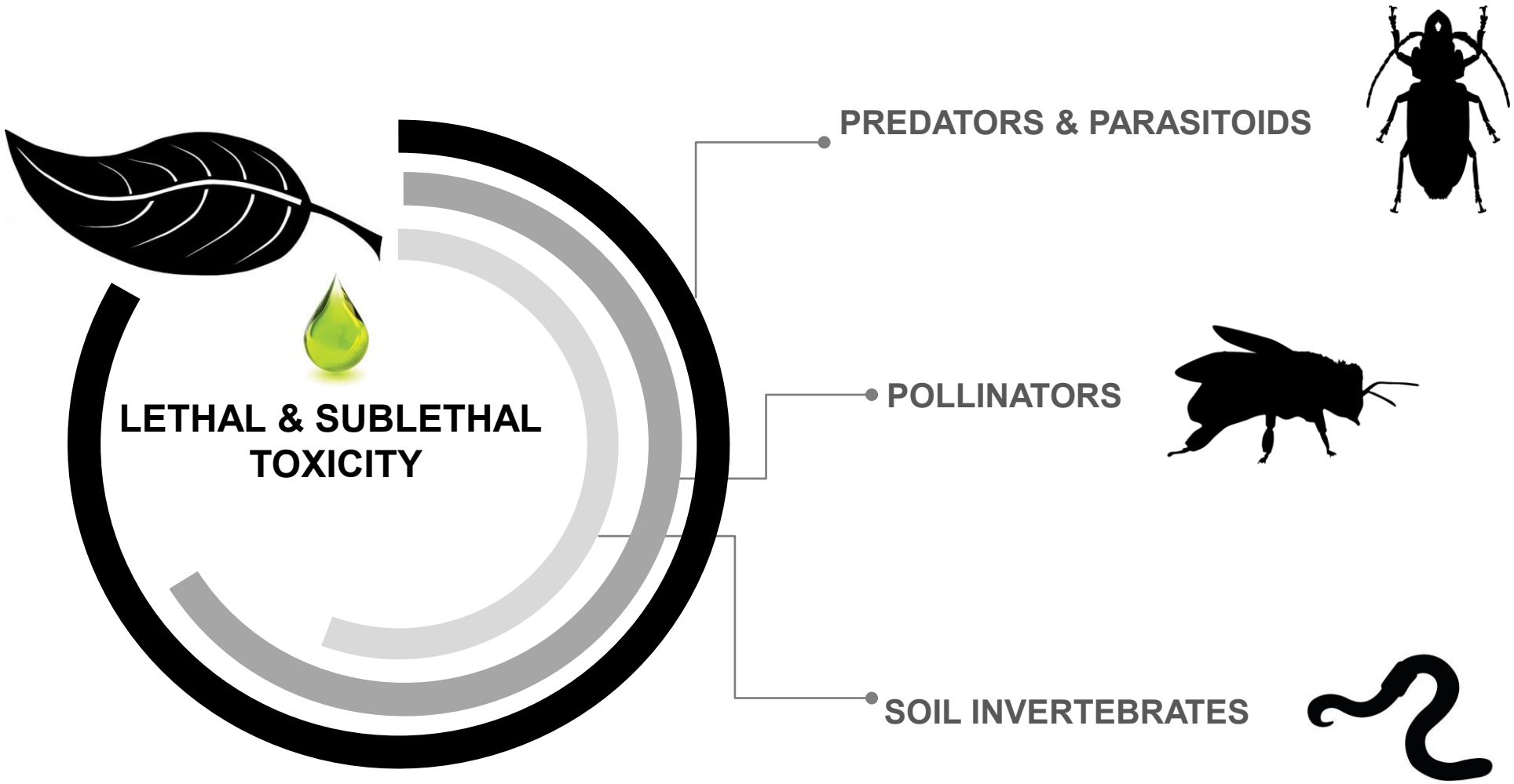
Non-target effects of essential oil-based biopesticides for crop protection: Impact on natural enemies, pollinators, and soil invertebrates

Highlights

- Literature on essential oils (EOs) as effective insecticides and acaricides is steadily growing
- The non-target impact of EOs include lethal and sublethal effects
- We analyzed literature on EO toxicity towards biocontrol agents, pollinators, and soil invertebrates
- The modes of action leading to EO toxicity on non-target species are scarcely studied
- In the final section, a research agenda outlining major challenges in the field is proposed

Graphical Abstract

ESSENTIAL OIL-BASED INSECTICIDES AND ACARICIDES



Invited Review for Biological Control

Non-target effects of essential oil-based biopesticides for crop protection: impact on natural enemies, pollinators, and soil invertebrates

Giulia Giunti [1], Giovanni Benelli [2,*], Vincenzo Palmeri [3], Francesca Laudani [3], Michele Ricupero [4], Renato Ricciardi [2], Filippo Maggi [5], Andrea Lucchi [2], Raul Narciso C. Guedes [6], Nicolas Desneux [7,*], Orlando Campolo [3]

[1] *Department of Pharmacy, University of Salerno, via Giovanni Paolo II 132, 84084, Fisciano (SA), Italy*

[2] *Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, 56124 Pisa, Italy*

[3] *Department of Agriculture, University "Mediterranea" of Reggio Calabria, Loc. Feo Di Vito, 89122, Reggio Calabria, Italy*

[4] *Department of Agriculture Food and Environment, University of Catania, via S. Sofia 100, 95123 Catania, Italy.*

[5] *Chemistry Interdisciplinary Project (ChIP), School of Pharmacy, University of Camerino, Via Madonna delle Carceri 9/B, 62032, Camerino, Italy*

[6] *Departamento de Entomologia, Universidade Federal de Viçosa, Viçosa, MG 36570-900, Brazil6*

[7] *Université Côte d'Azur, INRAE, CNRS, UMR ISA, 06000 Nice, France*

* Corresponding authors.

E-mail address: giovanni.benelli@unipi.it (G. Benelli)

E-mail address: nicolas.desneux@inrae.fr (N. Desneux)

Abstract

The control of arthropod pests of agricultural importance is increasingly difficult due to the quick development of resistance in the targeted pest populations coupled to their massive non-target lethal and sublethal effects. This fostered the progressive banning of active ingredients at international and national levels, making pest management challenging. Reliable and environmentally sustainable pest control tools are required. Botanicals, with special reference to plant essential oils (EOs), can represent a broad source of active ingredients to develop effective insecticides and acaricides for agricultural purposes. In this context, our review analyzed the literature currently available about the lethal and sublethal activity of EOs on non-target terrestrial invertebrates in agricultural settings, including biological control agents (predators and parasitoids), pollinators and soil non-target species. Even if EO-based insecticides and acaricides are generally considered safer from a non-target point of view, a number of detrimental effects have been noted on biological control agents, including negative effects on respiration rate, reduced predatory ability and reduced parasitization rates, among others. Examples of sublethal effects experienced by pollinators exposed to EO-based pesticides are the reduction in the movement speed and distance travelled, while the toxicity of EO-based products on soil invertebrates is limited. Of note, the modes of action leading to EO toxicity on non-target species are scarcely studied. Further research on long-term non-target effects of EO-based pesticides in the field is still needed.

Key words: biocontrol; Integrated Pest Management; lethal effects; sublethal effects; parasitic wasp; honeybee; bumblebee; stingless bee; earthworm

1. Introduction

The still growing widespread demand and use of synthetic pesticides in agriculture pose several risks in terms of ecotoxicology, as well as social issues. Therefore, the harmful effects of some molecules on human health, as well as the negative impact on the environment, induced the international and national regulators to ban or severely restrict the application of several synthetic insecticides (e.g., multiple banned organochlorine and organophosphate insecticides, and more recently neonicotinoids in Europe, Jactel et al., 2019). Most of the authorized chemical insecticides and acaricides are still neurotoxic, affecting the nervous system of arthropod pests; however, synthetic active ingredients, especially the earliest ones, may also threaten human health, warm-blooded animals as well as non-target arthropods species such as biological control agents (BCA) and pollinators (Weisenburger, 1993; Desneux et al., 2007; Casida and Durkin, 2013). Compared to first and second-generation pesticides, the toxicity of last-generation synthetic pesticides has generally increased towards aquatic invertebrates and pollinators, while the acute toxicity towards mammals and birds has been reduced, mainly due to their low application rates (Schulz et al., 2021). Scientists have been working on the development of viable alternatives to synthetic chemicals which can be less harmful to the environment, and both researchers and consumers are paying even close attention to bioactive plant active ingredients for developing new green pesticides. The development of plant-based biopesticides has also attracted increasing interest from the pesticide industry in recent years and the issues related to the formulation and toxicology of pesticides are usually not shared by industries, because they are considered proprietary information. On this basis, it should be assumed that the scientific literature, which is the basis for the scientific dissemination, is lacking in some hard-to-access knowledges.

Several plant extracts can act either as toxicants or repellents, as well as phagodeterrents, ovideterrents or growth regulators and may provide viable alternatives to traditional synthetic pesticides (e.g. Shah et al., 2020; Verheggen et al., 2022), since they are a valuable source of bioactive molecules (Campolo et al., 2018; Kavallieratos et al., 2021). Among the plant extracts proposed for

pest control, essential oils (EOs) are promising active ingredients for insecticidal formulations due to their worldwide availability and relative low cost and their presumed safety for human health and the environment (Isman, 2020; Li et al., 2022; Palermo et al., 2021). EOs are secondary metabolites produced by plants for a variety of purposes and they are involved in indirect plant defense mechanisms (i.e., against both biological and abiotic stress), and play a key role in signaling processes, including plant attractiveness toward beneficials and pollinators. EOs are produced by several plant species, i.e. the so-called aromatic plants belonging to a panel of botanical families such as Asteraceae, Apiaceae, Lamiaceae, Lauraceae, Myrtaceae, Verbenaceae, Geraniaceae, Zingiberaceae, Pinaceae, and others (Benelli et al., 2017; Pavela et al., 2021a, 2021b; Spinozzi et al., 2021). They are synthesized and eventually stored in secretory structures of epidermal or parenchymatic origin which are distributed in different plant parts or organs, such as roots, bark, leaves, seeds, fruits, bark, and tubers. Furthermore, EOs produced from the same plant but extracted from different organs can vary significantly both in terms of chemical composition and yield. Even when the same plant species is considered, the yield and composition of EOs may vary with the cultivated environment and the plant genetic background leading to the presence of different chemotypes within the same species (Pavela and Benelli, 2016).

EOs are phytocomplexes composed of a blend of substances (i.e., often more than 50), including terpenes (monoterpenoids and sesquiterpenoids), the most frequent constituents, but also aromatic (i.e., phenylpropanoids, aldehydes, alcohols, esters, etc.) and aliphatic compounds (i.e., alkanes, aldehydes, alcohols, ketones, esters) and others (i.e., polyacetylenes). It is quite common that a single compound accounts for more than 20% of a given EO; as an example, the relative content of D-limonene in orange EO exceeds 50% of total components (Buriani et al., 2020).

Because EOs are accumulated inside plant organs, they must be collected from plant tissues using different extraction techniques. The most common extraction techniques are hydrodistillation (HD), steam distillation, and cold pressing (CP). These sometimes are characterized by a variety of disadvantages, including low efficiency and yield, and degradation of some molecules (Reyes-Jurado

et al., 2014). Due to the above-mentioned limitations, several new techniques have been developed to effectively extract EOs, such as microwave-assisted extraction (MAE), which improves the production efficiency while reducing time and energy consumption during the process (Sawamura, 2011; Fiorini et al., 2020).

The EO characteristics (i.e., high volatility and biodegradability, low persistence in the environment) which make these phytocomplexes promising active ingredients for biopesticides, also limit their application as commercial plant protection products. These limitations reduce the possibility of the use of EOs as such and the difficulties in patenting the EO-based pesticide formulations have limited the spread of commercial formulations. Since these bioinsecticides are moderately commercialized and represent a restricted market at present, it can be assumed that EOs are unsuccessful control tools under field conditions. Nonetheless, field evaluations showed that EOs can be effective in some situations and can obtain pest control levels comparable to organo-synthetic pesticides (Isman et al., 2011; Smith et al., 2018). Despite the huge number of studies about EO bioactivity against pests, the main commercialization of bioinsecticides based on EOs dates over a decade ago in the USA and just 6-7 years in the EU (Isman, 2020). Commercially available formulations may contain a single EO or EO constituent, a mixture of different EOs, as well as a blend of synthetically produced terpenoids. These formulations and EOs in general are often acknowledged to be safe for the environment and human health based on the physicochemical properties of these compounds derived from their respective structures; however, few studies, compared to the large bibliography available about EOs toxicology against pest species, focused on the ecotoxicological impact of EOs and EO constituents against non-target species (Haddi et al. 2020; Turchen et al. 2020).

In a recent paper, Ferraz et al. (2022) reviewed the impact of both EOs and plant extracts on non-target organisms, namely microalgae, crustaceans, fishes, plants, and soil (micro)organisms; however, terrestrial invertebrate species, such as BCA and pollinators, were not considered. Natural enemies of crop pests, as well as pollinators, can directly contact with pesticides on sprayed crops

and contaminated nearby vegetation, and they can feed on treated plants/preys/hosts. Furthermore, soils can also be polluted by residues of pesticide applications due to drift phenomena, and the abundance and variety of invertebrate species in soils is a recognized bioindicator for environmental health and pollution, which should be accounted in agroecosystems (Burger, 2007). In this context, this review focuses on the main findings about lethal and sublethal effects of EOs against non-target terrestrial invertebrates in agriculture, including beneficial arthropods (i.e., predators, parasitoids, pollinators), as well as soil non-target species.

2. Invertebrate predators of crop arthropod pest species

Among beneficials playing a key role in biological control programs against several pests, predators are valuable control agents due to their ability to feed on and kill several to many individual prey during their lifetimes. Predatory beetles, flies, lacewings, true bugs, and predatory mites are just some examples of predators used in biological control programs. The side effects of EOs on these predators are quite variable depending on different parameters, including plant species, EO formulation, application technique, and non-target species and life stage (**Table 1**).

2.1. Lethal effects of EOs toward invertebrate predators

EOs are generally considered safe for non-target predators, because of their high mobility and their larger size compared to target species. In this regard, it is commonly acknowledged that higher doses of toxicants are needed to kill larger species or specimens, although there are exceptions. In contact toxicity tests on *Aphis punicae* Passerini (Hemiptera: Aphididae) adults, it was shown that LC_{50s} for various EOs were approximately four-fold lower than those estimated for *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) larvae (Sayed et al., 2022). Furthermore, *Satureja intermedia* C. A. Mey EO is a good candidate to develop plant-derived aphicides because of its toxicity against *Aphis nerii* Boyer de Fonscolombe (Hemiptera: Aphididae), coupled with its relative safety to the generalist predator *Coccinella septempunctata* L. (Coleoptera: Coccinellidae)

(Ebadollahi and Setzer, 2020). On the other hand, fumigation with four EOs toxic to aphid pests (*Mentha pulegium* L., *Mentha* x *piperita* L., *Ocimum basilicum* L., and *Citrus sinensis* (L.) Osbeck EOs) caused variable mortality on two coccinellid predator species, the seven-spotted ladybird *C. septempunctata* and the two-spotted ladybird *Adalia bipunctata* L. (Coleoptera: Coccinellidae) with distinctive selective toxicity ratios depending on the considered aphid species, coccinellid predator and EO (Kimbaris et al., 2010).

Bioactive botanical compounds can be more selective than commercial synthetic insecticides (Benelli et al., 2019c, 2018a; Pavela, 2018); as an example, the EO of *Lippia sidoides* Cham., (Verbenaceae) and its major compound thymol were less toxic than deltamethrin toward the predator *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae), a predator of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae); besides, deltamethrin led to quicker mortality to nymphs of *P. nigrispinus* (LT_{50}= 0.36 h) compared to EO (LT_{50}= 119 h) and thymol (LT_{50}= 93 h). Moreover, these botanical compounds acted against the pest faster than the synthetic insecticide (Lima et al., 2020). Similarly, dichlorvos was more toxic (LD_{50} 9.0×10^{-10} mg cm^{-3}) against *Orius strigicollis* Poppius (Hemiptera: Anthocoridae), compared with *O. basilicum* EO constituents, whose LD_{50} values ranged from 0.0127 to > 0.23 mg cm^{-3} (Kim et al., 2015).

Nevertheless, EO-based formulations are not always selective to predators of target species. As an example, LC_{50} values for *Vanillosmopsis arborea* Baker and *Lippia microphylla* Cham. EOs topically applied to *S. frugiperda* larvae were 172.86 mg mL^{-1} and 104.52 mg mL^{-1} respectively, but the lethal concentrations for the generalist predator *Euborellia annulipes* Lucas (Dermaptera: Anisolabididae), were similar or even lower (*V. arborea* LC_{50} = 160.2 mg mL^{-1}; *L. microphyla* LC_{50} = 134.67 mg mL^{-1}) (Alves et al., 2022). Furthermore, EOs can cause mortality of predators both by direct contact, as well as by ingestion of treated prey, as supported by the survival of *Podisus maculiventris* Say (Heteroptera: Pentatomidae) to *Curcuma longa* L. EO and its major components after topical application and ingestion of treated *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) larvae (Tavares et al., 2019). In some cases, EOs can be safe to adults and pre-imaginal stages of

predators while causing mortality of eggs, impairing egg hatching; this is the case of *Rosmarinus officinalis* L. EO, which caused low mortality rate toward *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) larvae, however having negative effects on the eggs hatching rate of the same species (Azimi Zadeh and Ahmadi, 2018).

The use of EOs as acaricides has also been studied in depth, since phytophagous mites are serious pests in greenhouse and field agricultural ecosystems. Among the EOs used for the control of mites, the one extracted from *Lippia sidoides* Cham. exhibited a good toxicity against *Tetranychus urticae* C.L. Koch (Acari: Tetranychidae) as well as a good selectivity towards the predator mite *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) (de Santana et al., 2021). This predatory mite was also more tolerant than the target pest to *Melissa officinalis* L. (Momen et al., 2014), *Piper aduncum* L., *Melaleuca leucadendra* L., and *Schinus terebinthifolius* Raddi EOs, as well as their binary blends (de Araújo et al., 2020), while it was sensitive to *P. marginatum* Jacq. EO (Ribeiro et al., 2016). Similar results were reported for *Typhlodromus ornatus* Denmark & Muma (Acari: Phytoseiidae), a naturally occurring generalist predatory mite in coconut plantations, which was not affected by sweet orange (*C. sinensis*) cv "Pera" EO at the lethal concentrations used against the target mite species *Aceria guerreronis* Keifer (Acari: Eriophyidae) (Brito et al., 2021). The selectivity of the tested EOs towards *T. ornatus* may be related to the biological, anatomical, and physiological differences between predators and their prey, such as the integument or presence of detoxifying enzymes (Sato et al., 2006; Tsolakis and Ragusa, 2008). Conversely, dos Santos et al. (2019) reported that the EO from *Lippia gracilis* Schauer was toxic both against the target species *Raoiella indica* Hirst (Acari: Tenuipalpidae) as well as against the predator mite *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae), since the LC_{50} (4.99 mg/mL) of the EO estimated for *R. indica* caused 48.33 ± 3.07% mortality to *A. largoensis*.

Within the same plant genus, EOs extracted from different plant species or chemotypes can have different efficacy toward target pests, as well as adverse effects on non-target organisms (Seixas et al., 2018a). Nevertheless, pennyroyal EO (*Mentha pulegium*) extracted from two different

chemotypes (i.e., major constituent pulegone or piperitone) revealed a good insecticidal activity against *Aphis gossypii* Glover (Hemiptera: Aphididae), *A. spiraecola* Patch (Hemiptera: Aphididae) and *T. urticae* (Acari: Tetranychidae) at 1000 µL/L of EO concentration in spray applications irrespective of the chemotype; the impact of both EOs on the polyphagous predator *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) was negligible (Papadimitriou et al., 2019). Similar results were highlighted by Ricupero et al., (2022) in which garlic EO based nano-emulsion revealed a significant toxicity against *Tuta absoluta* while no lethal effects were highlighted towards *N. tenuis* adults. On the other hands, the same formulation had a significant impact on the progeny produced by females allowed to develop on treated plants. Shaltoki et al. (2022) confirmed the negative effect of pennyroyal EO applications towards *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) eggs and first-instar larvae, affecting both survival and reproductive performances of the developed adult beetles.

Considering different closely related species, the evaluation of different *Citrus* peel EOs towards the generalist predator *N. tenuis* demonstrated a significant variability in terms of acute mortality and side-effects depending on the type of formulation, the EO used and the different residual times (Campolo et al., 2020). Moreover, exposure time is also a key factor determining the effects of insecticides on non-target species; indeed, the EOs extracted from *Artemisia sieberi* Besser, *Pelargonium graveolens* L'Hér., and *Ferula gummosa* Boiss. Showed similar toxicity against the pest *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Zandi-Sohani et al., 2018). Conversely, their effects on the generalist predator *Orius albidipennis* (Rueter) (Hemiptera: Anthocoridae) varied according to the EO and the exposure time, although the LC_{50} values against predators were significantly higher than those of target pest species (Zandi-Sohani et al., 2018). These results suggest that the compatibility of EO-based pesticides in organic agriculture can be improved through careful timing of treatment and release of natural enemies. Indeed, most of these substances exert their toxic activity only at high doses for a limited period after treatment and, in general, the toxicity towards natural enemies is significantly reduced with the aging of residues both toward generalist and specific

predators (Brito et al., 2021; Campolo et al., 2020, 2017). Although low persistence is a desirable trait in conventional pest management, the rapid degradation and volatility of EOs in the agroecosystems can limit their effectiveness against the target species and, at the same time, can be useful where natural enemies need to be protected.

The formulation of EO in organic solvent can also mitigate potential negative effects toward non-targets, while maintaining pesticidal activity (Abdel Kader et al., 2015); as an example, *Varronia curassavica* Jacq. (Boraginaceae) oil-in-water emulsion revealed a good insecticidal activity against the target pests *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and *T. urticae*, while it did not affect the survival of the generalist predator *Ceraeochrysa cubana* Hagen (Neuroptera: Chrysopidae), even when applied at the highest tested application rate (1%) (Andrade et al., 2021). Amer et al. (2016) evaluated the toxicity of *Laurus nobilis* L. EO and its commercial formulation Lauricide® on the predatory mite, *Typhlodromus negevi* Swirski and Amitai and *Phytoseiulus persimilis* Athias- Henriot (Acari: Phytoseiidae); predatory females were found to be more tolerant than *T. urticae* females to both materials, with LC_{50} values higher for the EOs (1.82×10^4 and 2.00×10^4 ppm for *T. negevi* and *P. persimilis,* respectively) compared to the formulation (0.28×10^4 and 0.40×10^4 ppm).

The evaluation of the efficacy of various conventional and biological pesticides against the prickly pear cactus cochineal *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) and their selectivity towards its natural predator *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) have been investigated by El Aalaoui et al. (2019). Among the tested insecticides, the Prev-am® commercial formulation based on d-limonene (the main compound of sweet orange EO) was effective in controlling *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) and, at the same time, showed a low impact on adults and larvae of the coccinellid predator (El Aalaoui et al., 2019). Similarly, Soares et al. (2019) demonstrated that the survival of the mirid predator *N. tenuis* is not affected by Prev-am® at various concentrations.

The susceptibility of predator species to EOs may be caused by physiological alterations. The EOs from *Mentha spicata* L. and *Melaleuca alternifolia* (Maiden & Betche) Cheel were used to

evaluate the effect of ingestion of treated prey by *P. nigrispinus*. Ático Braga et al. (2020) demonstrated that *M. alternifolia* EO administration caused an elongation of digestive cells, followed by cell lysis and tissue necrosis, while *M. spicata* caused just a reduction in the carbohydrate levels.

2.2. Sublethal effects of EOs toward invertebrate predators

Apart from acute lethal toxicity, EO administration can influence various life-history traits of non-target predators such as their reproductive performance and predatory ability, which are the most investigated biological parameters in this context and are designated sublethal effects (see Desneux et al., 2007 for a thorough review). EOs and their terpenoid constituents can affect the physiology of insects and mites in different ways and places, resulting in a disruption of reproductive processes such as oogenesis, vitellogenesis, maturation, and spermatocyte growth (Shaltoki et al., 2022). The LC_{50} and LC_{80} of the 'Pera' sweet orange EO, estimated for *A. guerreronis*, did not affect the population growth of the generalist predatory mite *T. ornatus* (Brito et al., 2021). In contrast, sublethal effects on fecundity and fertility were observed in *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) for d-limonene, while oregano EO affected only the fecundity of this green lacewing (Castilhos et al., 2018).

Predatory behavior can also be influenced by insecticidal and acaricidal treatments; the walking activity of *N. tenuis* adults exposed to leaves treated with synthetic pyrethroid (lambda-cyhalothrin) and *Citrus* EO-based biopesticide (Prev-am®) was significantly higher compared to the control treatment, while the predatory voracity was reduced by lambda-cyhalothrin and increased by Prev-am® treatment (Soares et al. 2019). Similarly, Passos et al., (2022) demonstrated that *N. tenuis* adults biological traits (fertility and orientation behaviour) were negatively affected by exposing the mirid to the tested EOs formulations (garlic, anise, fennel and lavender).

In contrast, Abdel Kader et al. (2015) evaluated the effect of *M. officinalis* EO and its commercial formulation (Melissacide®) against females of two predatory phytoseid mites, *Typhlodromips swirskii* (Athias Henriot) (Acari: Phytoseiidae), and *Neoseiulus barkeri* (Hughes)

(Acari: Phytoseiidae), showing that Melissacide® can reduce food consumption, while moderate effects were highlighted in the daily number of deposited eggs. Similarly, eggs of both predatory mites were not influenced by *L. nobilis* EO, while its formulation reduced oviposition and food consumption, also influencing the sex-ratio of the offspring (Amer et al., 2016). The effect of an EO can be species-specific; the exposure to *Siparuna guianensis* Aubl. EO did not affect the predatory abilities of *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae) but increased the abilities of *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) to prey upon *M. persicae* (Toledo et al., 2019). Similarly, *Ceraeochrysa caligata* B. (Neuroptera: Chrysopidae) larvae surviving exposure to *Citrus* EO exhibited higher predatory ability when faced with prey scarcity (Farias et al., 2020). Brügger et al. (2019) investigated the impact of lemongrass EO and its constituents against *P. nigrispinus;* the terpenoid constituents of lemongrass EO had a negative effect on respiration rate of the hemipteran predator, probably due to muscle paralysis, disruption of oxidative phosphorylation processes and dysregulation of the breathing activities, which could explain the reduced predatory ability. In addition, *P. nigrispinus* nymphs exposed to treated surfaces demonstrated irritability or repellency (Brügger et al., 2019).

Furthermore, EOs can play an important role to improve the efficacy and the accuracy of predators' activity. Liu et al. (2019) showed that *Coriandrum sativum* L., *Alpinia officinarum* Hance, *Manilkara zapota* (L.) P. Royen and *Nerium indicum* Mill. EOs, EO fractions, and two derived compounds, isocaryophyllene and *trans*-2-dodecenol, attracted both adults and nymphs of *Cyrtorhinus lividipennis* Reuter (Hemiptera: Miridae), predator of *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae). The ability of EOs or some of their compounds to lure natural enemies was investigated by several authors. As examples, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) laid more eggs in beans treated with limonene or β-caryophyllene than in control seeds (Alhmedi et al., 2010). Similarly, the green lacewing *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae) preferred as oviposition sites pecan branches treated with β-caryophyllene than untreated branches (Kunkel and Cottrell, 2007). Attractancy/repellency of EOs toward the spider

Pardosa pseudoannulata Boesenberg and Strand (Araneae: Lycosidae) was evaluated in choice tests using EOs of *Piper nigrum* L. and *Litsea cubeba* (Lour.) Pers., or their mixture as given cues, revealing that these EOs had no significant influence on the orientation of the predator while the mixture elicited its attraction (Farid et al., 2019).

3. Parasitoids of crop insect pest species

Parasitoids represent one of the best weapons among the BCA used against various pests. Their success is due to their effectiveness in intercepting the host, which is generally more sophisticated than that of predators, and their high efficacy in rapidly reducing the population density of the target host. One of the critical issues in the use of parasitoids is their susceptibility to pesticides, which are commonly used in organic agricultural systems as well (Biondi et al., 2015). Since EOs are considered eco-friendly tools for pest control, the belief has arisen that they can also be used in combination with the release of natural enemies, with special reference to parasitoids *prmis* (Monsreal-Ceballos et al., 2018). But is it real? The effects of EOs toward parasitoids are summarized in **Table 2**.

3.1.Lethal effects of EOs toward parasitoids

Research concerning the biological effects of EOs towards parasitoids showed contrasting results depending on *i*) the parasitoid species; *ii*) the EO used; *iii*) the host/parasitoid instars or *iv*) the administration technique.

Several EOs or EO constituents demonstrated a promising selectivity against key crop pests (Chiasson et al., 2004; Sümer Ercan et al., 2013; Yotavong et al., 2015). *Rosmarinus officinalis* EO and its major compounds had good larvicidal effect against *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), whereas they did not cause adult parasitoid mortality in topical application and ingestion bioassays (Trombin De Souza et al., 2021). The EOs from *Hyptis marrubioides* Epling and *O. basilicum* were classified as harmless according to the IOBC (International Organization for Biological Control) criteria against the parasitoid *Trichogramma pretiosum* Riley (Hymenoptera:

Trichogrammatidae), so they potentially could be used in *S. frugiperda* integrated pest management programs (Bibiano et al., 2022). Similarly, oregano, peppermint, and thyme EOs were more toxic to different instars of *Diaphania hyalinata* (L.) (Lepidoptera: Pyralidae) than toward its adult parasitoid, *Trichospilus pupivorus* Ferrière (Hymenoptera: Eulophidae), in residual contact toxicity trials, whereas the toxicity of ginger EO was comparable for both the pest and the natural enemy (Moreira Da Silva et al., 2020). Therefore, EOs are not always harmless for parasitoids, as reported by Zapata et al. (2016), who evaluated the toxicity of *Laurelia sempervirens* (Ruiz & Pav.) Tul. (Atherospermataceae) EO against adult *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) (LC_{50} = 3.77 µL L^{-1} air) and the parasitoid *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae) (LC_{50} = 0.86 µL L^{-1} air).

Despite the non-target toxicity highlighted for some EOs, these botanicals are usually less toxic than commercial synthetic insecticides, as reported by Yi et al. (2016), who demonstrated that a mixture of 21 *Lavandula angustifolia* Mill. EO constituents was ~1,430 times less toxic than dichlorvos against *Cotesia glomerata* (L.) (Hymenoptera: Braconidae), a parasitoid of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), in spray application. However, *C. glomerata* remained more susceptible than its host to several EO fumigations (Yi et al., 2007).

Plant species and EO chemical characteristics deeply influence the toxicity toward parasitoids; *Habrobracon hebetor* Say (Hymenoptera: Braconidae), natural enemy of several Lepidoptera, was susceptible to *Foeniculum vulgare* Mill. (LC_{50}=0.48 µL L^{-1}) and *O. basilicum* EOs (LC_{50}=0.84 µL $^{-1}$), while *Achillea millefolium* L. (LC_{50}=1.68 µL L^{-1}) and *Zataria multiflora* Boiss EOs (LC_{50}=1.04 µL L^{-1}) were less toxic (Ahmadpour et al., 2021). Furthermore, LC_{50} values for *R. officinalis* and *Salvia officinalis* L. EOs against this braconid species are 4.15 and 18.36 µLL^{-1} of air, respectively. In addition, EOs extracted from five species of the genus *Piper* were tested against the pupal parasitoid *Trichopria anastrephae* Lima (Hymenoptera: Diapriidae), natural enemy of *D. suzukii*, but these EOs caused low parasitoid mortality (< 20%) both through ingestion and topical application (Trombin de Souza et al., 2020).

Different administration techniques can determine various degrees of selectivity. As an example, the application of different EOs as fumigants towards adults of the egg parasitoid *Trissolcus basalis* Wollaston (Hymenoptera: Scelionidae) highlighted a good selectivity of the tested EOs, while the same EOs were not selective in residual contact toxicity trials (Werdin González et al., 2013). Three EOs [*Lippia origanoides* Kunth, *Cymbopogon winterianus* Jowitt ex Bor, *Cymbopogon citratus* (DC.) Stapf] showed selectivity for the parasitoid *T. pretiosum* in residual contact toxicity experiments, resulting in a LC_{50} of 0.43%, 0.15% and 0.12% for *L. origanoides*, *C. citratus* and *C. winterianus,* respectively (Sombra et al., 2022). Time interval between EO treatment and parasitoid release can be a key factor for EO selectivity. The parasitoid wasps *Dinarmus basalis* (Rond.) (Hymenoptera: Pteromalidae) and *Triaspis luteipes* (Thomson) (Hymenoptera: Braconidae), developing on bruchid host larvae, were tested for their susceptibility to *Artemisia herba-alba* Turra and *A. campestris* L. EOs. *Dinarmus basalis* was susceptible in fumigation trials while parasitoids released 6 days after treatment had reduced negative effects, as well as *T. luteipes,* whose emergence was just slightly reduced (Titouhi et al., 2017). Similarly, Ketoh et al. (2005, 2002) demonstrated that EO vapors and residues (6 days from treatment) can be highly toxic toward adult *D. basalis*, and all developmental stages of this parasitoids were very susceptible to *Hyptis spicigera* Lam and *H. suaveolens* (L.) Kuntze EOs (Sanon et al., 2011).

Parasitoid instars can be differentially affected by EO administration according to their biology (i.e., larval-pupal/egg and endo/ecto-parasitoids) because they can be protected by the parasitized host. Adults of *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae) a pupal parasitoid of *D. suzukii,* were susceptible to EO vapors extracted from *Mentha arvensis* L., whereas the immature instars were unaffected by this EO, probably because they are developing within the host puparia which can protect the parasitoid from toxicants (Gowton et al., 2020).

Similar to pupal cases, the egg chorion may protect parasitoids from the negative impacts of EOs targeting their host pests. Indeed, *P. aduncum* EO applied against *Euschistus heros* (F.) (Hemiptera: Pentatomidae), a key soybean pest, did not affect the emergence of either *Telenomus*

podisi (Ashmead) (Hymenoptera: Platygastridae) and *Trissolcus urichi* (Crawford) (Hymenoptera: Platygastridae) egg parasitoids (Turchen et al., 2016). Conversely, the preimaginal stages of *Trichogramma embryophagum* (Hartig) (Hymenoptera: Trichogrammatidae) and *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae), developing inside *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs, suffered reduced emergence rate due to the application of *Ferula assafoetida* L. EO (Poorjavad et al., 2014). Several EO compounds can penetrate the egg chorion acting as insecticides against immature stages of developing natural enemies, such as *Trichogramma galloi* Zucchi (Hymenoptera: Trichogrammatidae), an egg parasitoid of *Diatraea saccharalis* Fabricius (Lepidoptera: Crambidae) (Parreira et al., 2018). Indeed, *Allium sativum* L., *Carapa guianensis* Aublet, *C. sinensis* (L.) Osbeck, *Origanum vulgare* L., *Syzygium aromaticum* (L.) Merr. & L.M.Perry EOs reduced the parasitoid F_1 emergence rates by more than 30%, while *Zingiber officinale* Roscoe EO can drastically reduce the emergence rate (i.e., around 90%) when the parasitized eggs were treated during parasitoid pupal stage (Parreira et al., 2018). On the other hand, the trichogrammatid parasitoid *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) is less susceptible to the previously listed EOs, except for *C. guianensis* EO (27.3% decrease of emerged adults) and *Z. officinale,* which could completely nullify the emergence of adult parasitoids (Parreira et al., 2019).

Most of the EOs-based insecticides or acaricides are formulated by using single compounds or oils, even though mixtures of different EOs, or compounds, can improve their efficacy against target pests while conserving natural enemies. A combination of *Cedrus atlantica* (Endl.) Manetti ex Carrière, *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S and *C. citratus* (Stapf.) EOs was effective against *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) larvae without causing any deleterious effects on the the emergence rate of the koinobiont larval-pupal endoparasitoid *Psyttalia concolor* (Szépligeti) (Hymenoptera: Braconidae) (Alves et al., 2020). The use of the aforementioned EOs at 1.8% of application rates highlighted that, between the adult females of both species, *P. concolor* was more tolerant than the medfly, as the LD_{50} value estimated for *P. concolor* was 6.5-fold higher than

C. capitata one (Alves et al., 2020). Similar results were reported by Benelli et al. (2013) who found that *M. alternifolia* EO was more toxic to *C. capitata* than to its parasitoid *P. concolor* in contact, fumigation, and ingestion toxicity trials.

3.2. Sublethal effects of EOs toward parasitoids

Compared to predator species, more studies present results about sublethal effects of EO on the life-history traits of parasitoids, mainly focusing on the parasitization ability of the adult females. Parreira et al. (2019) identified two EOs (*Allium sativum* and *Carapa guianensis*) decreasing the parasitism rate of *T. pretiosum* females (33 and 70%, respectively), indicating these EOs as slightly harmful (class 2) in relation to parasitism according to IOBC toxicity categories. Furthermore, *Leptospermum petersonii* F.M. Bailey EO appeared harmless to *T. pretiosum*, since both the oviposition rate and the adult survival were not affected by the EO treatments (Purwatiningsih et al., 2012). In contrast, the closely-related species *T. galloi* reduced its parasitization ability (between 30 to 79%) in F_1 and F_2 parasitoid generations after treatments with *A. sativum*, *C. guianensis*, *C. sinensis*, *Azadirachta indica* A. Juss. and *O. vulgare* EOs (Parreira et al., 2018). Nevertheless, the EO from *Z. officinale* completely nullified the parasitism rate of *T. pretiosum* on eggs of *E. kuehniella*, suggesting a strong repellent activity of this EO toward the parasitoid females (Parreira et al., 2019).

Nevertheless, some EOs have no effect on the parasitism rate of parasitoid species; as an example, EOs from *O. vulgare* and *Thymus vulgaris* L. were selective fumigants, evoking no change in parasitoid behavior, and one week-old residues were safe also to *T. basalis* adults (Werdin González et al., 2013). Similarly, *P. concolor* treated with a mixture of EOs at 1.8% presented no deleterious effects on the percentage of parasitized *C. capitata* larvae, whereas parasitism rate decreased during the 2 first days after treatment at the highest concentration tested (4.8%) (Alves et al., 2020). Furthermore, the differences of acute toxicity among EOs do not always correspond to their side-effects (Sombra et al., 2022); *A. millefolium* and *Z. multiflora* EOs had lower LC_{50} values

on parasitoid wasps *H. hebetor* than *F. vulgare* and *O. basilicum* EOs, although LC_{30} values affected the fecundity and fertility of treated wasps similarly for all the tested EOs (Ahmadpour et al., 2021).

The reproductive ability of *E. formosa* was significantly affected by the administration of low doses (i.e., lower than LC_{50} for target pests) of *L. sempervirens* EO, but this treatment also decreased the host parasitism ability and the total number of offspring produced by each parasitoid female (Zapata et al., 2016). *Zingiber officinale* EO was able to reduce the *T. galloi* offspring production of F_1 and F_2 generations between 30 and 99%, showing a transgenerational effect, while this EO had little influence on the female parasitism rate (Parreira et al., 2018). Nevertheless, the sex ratios of the two *T. galloi* generations were neither affected in *T. galloi* nor in *T. pretiosum* (Parreira et al., 2019, 2018). Under laboratory conditions, *Eugenia uniflora* L. EO was effective against different life stages of *Thaumastocoris peregrinus* (Carpintero & Dellapé) (Hemiptera: Thaumastocoridae), but this EO was harmful towards the egg parasitoid *Cleruchoides noackae* Lin & Huber (Hymenoptera, Mymaridae), having also transgenerational effects (Stenger et al., 2021). The fertility life table parameters of *Trichogramma embryophagum* (Hartig) (Hymenoptera: Tricogrammatidae) and *Trichogramma evanescens* Westwood (Hymenoptera: Tricogrammatidae) were assessed after treatments with *F. assafoetida* EO, and female longevity, total number of offspring, number of female offspring per female (sex ratio), progeny wing abnormality in the progeny and developmental time were negatively altered for both species when parasitoid females were treated with very low EO concentrations (i.e., LC_{01}) (Poorjavad et al., 2014). Furthermore, the same research also investigated the reproductive behavior of *Trichogramma* spp., which can influence the parasitoid performances. Poorjavad et al. (2014) noted that mating success and occurrence were affected by EO, as well as the duration of copula were reduced; on the other hand, the time spent by males in mating searching behavior increased, highlighting some impairments caused by EO administration.

Apart from reproductive impairments, other side-effects can involve the developmental time of both treated parasitoid and their offspring. *Dinarmus basalis* females almost halved the parasitism rate on bruchid larvae treated with *Hyptis* spp. EOs, and the eclosed larvae presented a significantly

extended pre-imaginal developmental time (Sanon et al., 2011). Some EOs can thus influence population dynamic parameters such as: population growth rate (r or λ), net reproductive rate (R_0) and gross reproductive rate (GRR) of parasitoid species (Ahmadpour et al., 2021; Razmjou et al., 2018). As an example, Asadi et al. (2018) reported that the EOs of *R. officinalis* and *S. officinalis* can negatively affect several parameters of the parasitoid *H. hebetor*, including adult longevity, fecundity and fertility, population growth rate, gross and net reproductive rates, mean generation and doubling time, survival and death rate and cohort survival rate. Besides, also adult longevity can be reduced; fumigation with clove EO and geranial (0.5µL $50mL^{-1}$ of air) caused above 90% reduction in egg hatchability and life span of *H. hebetor* adults (Moawad et al., 2015). Similarly, the longevity of *T. pretiosum* females (i.e., both directly treated with EO or from F_1 generation) was almost halved in presence of *A. sativum* or *M. piperita* EOs (Parreira et al., 2019). Yotavong et al. (2015) noted that thymol could influence some biological parameters of the progeny of the parasitoid *C. plutellae*, at sublethal doses, like the emergence rate and the larval-pupal developmental time. However, there was no impact on detoxification enzymes (cytochrome P450 and carboxylesterase activities) (Yotavong et al., 2015).

Lastly, sublethal concentrations of EOs (LC_{30}) can cause consequences in the digestive system of the parasitoid *H. hebetor*, decreasing the enzymatic activity, but not the protein content, in this parasitoid wasp (Asadi et al., 2021).

4. Insect pollinators

EOs are very attractive products for pest control because they have low environmental persistence and mammalian toxicity (da Silva Sá et al., 2022; Campolo et al., 2018; Isman, 2017); however, research on natural products with insecticidal activity needs to also evaluate the bioactivity towards key groups of non-target insects, such as pollinators, which have been little explored (Turchen et al., 2020). During the flowering growth stage, many crop plants are important nectar and pollen sources for pollinator insects, which frequently visit the crops to collect pollen, nectar and

resins. This aspect highlights the need to assess the selectivity of EOs to these insect species because to date few studies focused on the bioactivity of these botanicals toward pollinators.

Despite the high insecticidal activity of *C. citriodora* EO against *Ascia monuste* (Godart) (Lepidoptera: Pieridae) (LD_{50} = 20.61 µg/mg) and its selectivity toward the predatory ant *Solenopsis saevissima* (Smith) (Hymenoptera: Formicidae), this EO caused high mortality among *Tetragonisca angustula* (Latreille) (Hymenoptera: Meliponini) adult forager bees, an important generalist pollinator species in tropical regions (Ribeiro et al., 2018). Similarly, *Artemisia annua* L. EO is a promising bioinsecticide against *D. hyalinata*, causing a low mortality against the predator ant *S. saevissima* (42 %), while significant toxicity was demonstrated toward the pollinator bee *T. angustula* (74%) (Seixas et al., 2018b). Therefore, the use of these EOs when the plants are in the flowering stage and constantly visited by bees, should be avoided. The absence of physiological selectivity of EOs, similarly to many synthetic commercial insecticides, does not preclude their use, although it should be considered under open field conditions. Nevertheless, some botanical extracts demonstrated good selectivity against stingless bees. In contrast with the previous results, when adult stingless bees, *Nannotrigona aff. testaceicornis* (Lepeletier) (Hymenoptera: Meliponini), were exposed to synthetic insecticides, *L. sidoides* EO or its major compounds in contact toxicity trials designed to evaluate the lethal and sublethal (i.e., locomotion and flight orientation) effects, the EO and its constituents demonstrated the lowest acute toxicity to forager worker bees, producing no effects on their locomotion and orientation ability (Matos et al., 2021). Furthermore, the authors reported that *N. testaceicornis* avoided *L. sidoides* EO and its major constituent thymol in arena trials, suggesting that this non-target species was repelled by the EO presence (Matos et al., 2021).

Consistent with the toxic activity reported for *T. angustula,* EOs can also impact the survival and behavior of the honeybee *Apis mellifera* L. (Hymenoptera: Apidae). Honeybees are beneficial and economically important insects, having a major impact on crop production because they represent 80% of insect pollinators, apart from the market for honey and beeswax. *A. mellifera* is a recognized bioindicator species since it is very sensitive and greatly affected by environmental changes and

pollutants, as well as by pesticide presence (Burger, 2006). Melo et al. (2018) reported that *L. gracilis* EOs and their major compounds (i.e., thymol and carvacrol) were effective against the target species *D. hyalinata*; however, these EOs were not selective to *A. mellifera* L. nor to *Polybia micans* Ducke (Hymenoptera: Vespidae), because in topical toxicity trials these botanicals (i.e., applied at the LD_{80} for *D. hyalinata*) caused significant mortality (> 80%) for both non-target species (Melo et al., 2018).

In bees, susceptibility towards an EO appears to be influenced by the exposed species rather than the EO. *Apis mellifera* foragers exposed to ginger, mint, oregano, and thyme EOs were less tolerant than *Trigona hyalinata* (Lepeletier) (Hymenoptera: Apidae) foragers (da Silva et al., 2020). Conversely, oregano and thyme EOs applied at sublethal doses had negative impact on the distance traveled, the movement speed and the number of stops by the stingless bee whereas, on *A. mellifera* foragers only oregano EO showed similar effects (da Silva et al., 2020). The walking activity of *A. mellifera* was negatively affected by eucalyptus EO, as well as neem seed kernel oil, which also showed a repellent effect towards honeybee foragers (Xavier et al., 2015).

On the other hand, some EOs (eucalyptus, camphor) or single compounds (i.e., thymol and menthol) are commonly used in commercial acaricide formulations (i.e., ApiLife Var® and Apiguard®) for *Varroa destructor* (Anderson & Trueman) (Mesostigmata: Varroidae) control, despite some moderate sub-lethal effects towards honeybees may raise some questions about their presumed complete harmlessness. Gashout et al., (2015) reported that among different EO compounds tested against the varroa mite, thymol and menthol had the lowest and the highest LC_{50} against both adult bees and larvae, respectively (adults: 210.3 and 523.5 µg/bee; larvae: 150.7 and 382.8 µg /larva). Furthermore, low concentration of EOs or single compounds (i.e., thymol and carvacrol) may also impact on the physiology of honeybees, mainly at nervous system level by causing an increase of acetylcholinesterase and glutathione S-transferase activities (Clavan et al., 2020), as well as EO compounds can be accumulate in their bodies by both adult bees and larvae (Sammataro et al., 2009).

In the last decade, nanotechnologies strongly influenced research on the formulation of novel insecticides, both synthetic and natural (de Oliveira et al., 2014; Vurro et al., 2019). Acute toxicity of

peppermint EO and its alginate-based nanoemulsion were recently evaluated against worker bees in oral and contact toxicity trials by Youssef and Abdelmegeed (2021); nanoemulsion was more toxic on *A. mellifera* than their crude materials both in contact (LC_{50} = 5471.13 and 11,895.65 ppm, respectively) and oral toxicity trials (LC_{50} = 2629.85 and 4246.84 ppm, respectively). Furthermore, both nanoemulsions and crude EO have biochemical and physiological effects on honeybee workers, altering amylase, total protein, and lipid contents (Youssef and Abdelmegeed, 2021).

5. Soil invertebrates

Among soil invertebrates, most of the studies aimed at the evaluation of the side-effects of EOs used earthworms as the bioassay species. Among this group, ecotoxicology tests mainly involved the non-target species *Eisenia fetida* (Savigny) (Haplotaxida: Lumbricidae)s (e.g., Kang et al., 2022; Nenaah et al., 2022; Pavela et al., 2019; Sánchez-Gómez et al., 2022; da Silva Sá et al., 2022). The acute toxicity of EOs against target crop pests and the non-target earthworm *E. fetida* are presented in **Table 3.**

Commonly the EOs have little to no effects against this non-target species; as an example, *Stevia rebaudiana* (Bertoni) EO was effective against the aphid *Metopolophium dirhodum* (Walker) (Hemiptera: Aphididae), while it had no effect towards non-target *E. fetida* adults (Benelli et al., 2020b). Similarly, Pavela et al. (2020b) demonstrated that the EOs extracted from *Oliveria decumbens* Vent., *Thymus daenensis* Celak *Satureja sahendica* Bornm., *S. khuzistanica* Jamzad and *S. rechingeri* Jamzad, effective insecticides against both moth and larvae of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), were slightly toxic towards *E. fetida* when applied at 200 mg kg^{-1} of soil, while the positive control α-cypermethrin induced complete mortality at a very low concentration (0.1 mg x kg^{-1} of soil) (Pavela et al., 2020b).

The commercial insecticide α-cypermethrin had a stronger impact on the survival of earthworms compared to several EOs, which appears to selectively favor *E. fetida* (Benelli et al., 2020a, 2019a, 2019b, 2019d, 2018b; Pavela et al., 2020a; Žabka et al., 2021). Similarly, two

organophosphate insecticides (i.e., monocrotophos and temephos) had a stronger impact on the survival, developmental rate, weight, and enzymatic activity of two earthworms *E. fetida* and *Eudrilus eugeniae* (Kinberg) (Haplotaxida: Eudrilidae) than the EO extracted from *Piper betle* L.; the LC_{50} observed for the two organophosphates were at least 775-fold lower than that estimated for the EO (Vasantha-Srinivasan et al., 2018, 2016). Furthermore, monocrotophos and temephos added in the soil repelled both earthworm species, whereas *P. betle* EO was attractive (Vasantha-Srinivasan et al., 2018). Similar results were also described by Murfadunnisa et al. (2019) who noted that *Sphaeranthus amaranthoides* Burm. f. (Asteraceae) EO caused no toxicity against *E. euginae* at the maximum dose of 1000 and 1500 ppm, while the synthetic chemical monocrotophos heavily affected the earthworm survival.

The formulation of the EO into nano-pesticides might influence target, as well as non-target bioactivity; the *Deverra tortuosa* (Desf.) DC. EO-based nanoemulsion exhibited an increased contact bioactivity ($LC_{50} = 10.3$ µg cm^{-2}) compared to crude EO ($LC_{50} = 23.1$ µg cm^{-2}), but both the tested products were safe toward the non-target earthworm *E. fetida* (Almadiy et al., 2022).

Aside from earthworms, the side-effects of eighteen EOs have been tested on adults of the soil collembolan *Proisotoma minuta* Tullberg (Collembola: Isotomidae), highlighting adverse effects in fumigation bioassays (Lee et al., 2002). Organic certified EO-based pesticides could also indirectly affect the presence of collembolan species, *Protaphorura fimata* Gisin (Poduromorpha: Onychiuridae), by repelling them from treated soils (Joseph, 2018). Furthermore, the EO from *Eucalyptus globulus* Labill. reduced the reproduction of the collembolan *Folsomia candida* Willem (Collembola: Isotomidae) ($EC_{50} = 35.0$ mg/kg), and the attractiveness of food toward both *F. candida* and the isopod *Porcellio dilatatus* Brandt (Isopoda: Oniscidae) (Martins et al., 2013).

6. Challenges for future research

Due to regulatory restrictions on conventional pesticides and consumer awareness of their deleterious effects on health and the environment, the demand for biopesticides is expected to constantly increase

in the next years; therefore, the ecotoxicological evaluation of this kind of pesticides is fundamental to understand their environmental impact. Nowadays, few studies, compared to the huge amount of research on EO bioactivity against crop pests, focused on the side effects toward natural enemies. Knowledge about non-target effects is needed to boost the large-scale industrial production of EO-based pesticides but also due to regulatory strictness. However, it is quite surprising that a very limited number of papers tested the side effects of commercial biopesticides containing EO as active ingredients, that have been on the market for at least a decade. Indeed, these commercial products might be used by farmers for integrated pest management programs involving biopesticides and BCA; nevertheless, the compatibility and economic sustainability of these two techniques should be addressed before suggesting their coupled application.

Generally, despite the usual lower efficacy of botanicals compared to conventional pesticides, the use of botanicals may be a valid alternative in terms of crop yields. Indeed, crops can tolerate a certain amount of pest damage and the selectivity of plant-based pesticide can ensure pest reduction through conservation of natural enemies and non-target species (Tembo et al., 2018). The selectivity of botanicals, including EOs-based pesticides, can be obtained following different paths such as: (i) timing of pest treatment; (ii) timing of natural enemies' release; (iii) correct choice of pesticide formulation according to the target pest and beneficials; iv) use of different types of formulations (e.g., nano vs. traditional). A holistic view of pest control that considers plant protection, environment, human health, and economic aspects will be able to facilitate the integration of biopesticides into agro-ecologically sustainable crop production systems.

From a commercial and marketing standpoint, only those effective EOs coming from plants which are cultivated on a large scale and that are obtainable in middle-high yield (> 1% on a dry weight basis; the price of an EO is inversely linked to the yield), thus offering a cost-effective raw material (often derived from cultivation waste), should be used for agrochemical industries. To improve the latter parameter, new effective extraction techniques (e.g., MAE, enzyme-assisted distillation, etc.) capable of boosting the release of EO constituents from the plant secretory structures

should be more explored in the future. From a registration perspective, the EOs which are generally recognized as safe (GRAS) from the principal authorities (i.e., FDA, EFSA, EPA, etc.) or are derived from plants with documented use as a food (so that they do not pose particular risk from their usage) should be preferred to the ones coming from plants subjected to some restrictions (e.g., toxic plants). Finally, more research is needed on the development and evaluation of the ecotoxicological effects of nanocarriers (e.g., micro- and nano-emulsions, nanoparticles made with plant polymers, liposomes, protein baits) able to incorporate these EOs and spread them on crops in an eco-sustainable way (Pavoni et al., 2019; Sanchéz-Gomez et al., 2022).

To date very few studies evaluated the impact of EO and their formulations toward pollinators; this aspect is crucial to understand the ecological impact of biopesticides in the fields, but it seems quite neglected by scientists. Pollination and pollinator losses are key topics in modern agriculture, as well as from an ecological point of view. Future studies should focus on the possible side effects of EOs toward these species to evaluate their eco-safety potential.

The modest number of studies exploring non-targeted effects of EO-based pesticides also share shortcomings common in studies with conventional insecticides, despite recent shift in that regard relative to the latter. Two important shortcomings in such studies merit particular attention: (i) the common assumption of a monophasic response with an increase in EO dose or concentration, and (ii) the study focus on isolated species. The first shortcoming neglects the possibility of biphasic concentration-response taking place, consistent with the hormesis phenomenon, in which exposure levels below the no-observed-adverse-effect-level (sub-NOEL) lead to a stimulatory response potential benefit to the exposed organism (e.g., non-targeted species) (Agathokleus and Calabrese, 2020; Belz and Duke, 2022). The potential importance of this phenomenon for pest management and environmental impact has been increasingly recognized for a broad range of anthropogenic stressors, including insecticides (Guedes and Cutler, 2014, Guedes et al., 2016, 2022), but largely neglected for plant-based compounds, such as EO-based pesticides (Haddi et al., 2020).

The second shortcoming on the current studies is the focus on isolated species, which although understandable based on a cost-effective experimental standpoint, neglect the fact that isolated species do not exist in natural environments and species interactions are prevalent. Thus, more realistic studies exploring the invertebrate communities are necessary not only to ascertain the field efficacy of EO-based formulations, but particularly to assess their potential non-target impact cascading from directly exposed targeted species to potentially directly and indirectly affected non-target species (Cutler et al. 2022; Guedes et al. 2016, 2022b). Conceptual frameworks such as the stress-response pathway are useful in that regard, although still underexplored even for the assessment of environmental impacts of conventional pesticides (Guedes et al., 2017). Thus, the rethinking and expanding of the scope of studies with EO-based insecticidal and acaricidal formulations is a need worth pursuing.

Therefore, national and international regulators are now paying more attention about the ecotoxicological impact of pesticides, including biopesticides based on plant-borne a.i., to ensure their environmental safety. In the last decades, the authorization process of botanicals has been greatly facilitated in the USA, by instituting exemptions from the normal regulatory approval process required for synthetic pesticides to certain EOs and their major constituents (Isman 2020). A similar approach has been used also by EU legislators, although with many more limitations and far less success (Vekemans & Marchand, 2020). In this regard, it should be kept in mind that the European legislation concerning plant protection products (PPP) (regulation (EC) N° 1107/2009) is quite unclear about the definition of PPP admitted in organic agriculture, botanical-based products included, thus the registration of green/biopesticides often faces insurmountable obstacles throughout the whole authorization process as a consequence (Vekemans & Marchand, 2020). In this scenario, research on non-target impact of botanical-based pesticides may improve the knowledge and the awareness about their ecotoxicological safety both among companies and industries, as well as within the regulator agencies, promoting and supporting further registration and commercialization.

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CRediT author statement

Conceptualization: Giulia Giunti, Giovanni Benelli, Orlando Campolo. **Writing - Original Draft:** Giulia Giunti, Giovanni Benelli, Michele Ricupero, Raul Narciso C. Guedes, Orlando Campolo. **Writing - Review & Editing:** Vincenzo Palmeri, Francesca Laudani, Renato Ricciardi, Filippo Maggi, Andrea Lucchi, Nicolas Desneux.

Table 1. Lethal and sub-lethal effects of essential oils (EO) toward non-target predator species.

Plant family	Plant EO	Non-target species	Target pest	Exposure route*	Non-target species life stage #	Endpoint	Lethal effects §	Sub-lethal effects §	References
Amaryllidaceae	*Allium sativum*	*Nesidiocoris tenuis*	*Tuta absoluta*	*R*	*A*	Survival; Fertility	-	+	Ricupero et al. 2022
Apiaceae	*Coriandrum sativum*	*Cyrtorhinus lividipennis*	*Nilaparvata lugens*	*R*	*N, A*	Attractivity; Orientation		+	Liu et al., 2019
	Ferula gummosa	*Orius albidipennis*	*Bemisia tabaci*	*F*	*A*	LC_{50} = 3.467 µL L^{-1}	-		Zandi-Sohani et al., 2018
	Heracleum persicum	*Hippodamia variegata*	*Rhopalosiphum padi, Sitobion avenae, Schizaphis graminum, Metopolophium dirhodum*	*I*	*E, N*	Mortality; Life history traits	-	+	Shaltoki et al., 2022
Apocynaceae	*Nerium indicum*	*Cyrtorhinus lividipennis*	*Nilaparvata lugens*	*R*	*N, A*	Attractivity; Orientation		+	Liu et al., 2019
Asteraceae	*Achillea millefolium*	*Hippodamia variegata*	*Rhopalosiphum padi, Sitobion avenae, Schizaphis graminum, Metopolophium dirhodum*	*I*	*E, N*	Mortality; Life history traits	-	+	Shaltoki et al., 2022
	Artemisia sieberi	*Orius albidipennis*	*Bemisia tabaci*	*F*	*A*	LC_{50} = 0.621 µL L^{-1}	+		Zandi-Sohani et al., 2018
	Vanillosmopsis arborea	*Euborellia annulipes*	*Spodoptera frugiperda*	*T*	*N*	LD_{50} = 160.2 mg mL^{-1}	+		Alves et al., 2022
Boraginaceae	*Varronia curassavica*	*Ceraeochrysa cubana*	*Myzus persicae, T urticae*	*R*	*N*	Mortality	-		Andrade et al., 2021
Brassicaceae	*Brassica nigra*	*Podisus maculiventris*	*Spodoptera exigua*	*T, I*	*N, A*	Mortality	-		Tavares et al., 2019
Euphorbiaceae	*Croton grewioides*	*Neoseiulus californicus*	*Tetranychus urticae*	*R, I*	*A*	LC_{50} = 3.26 µL mL^{-1}	-		de Santana et al., 2021
	Croton rhamnifolioides	*Neoseiulus californicus*	*Tetranychus urticae*	*R, I*	*A*	LC_{50} = 1.14 µL mL^{-1}	-		de Santana et al., 2021
Geraniaceae	*Pelargonium graveolens*	*Orius albidipennis*	*Bemisia tabaci*	*F*	*A*	LC_{50} = 0.954 µL L^{-1}	+		Zandi-Sohani et al., 2018
Lamiaceae	*Melissa officinalis*	*Neoseiulus barkeri*	*Tetranychus urticae*	*T*	*E, A*	Mortality; Food consumption; Fecundity	+	+	Abdel Kader et al., 2015
		Typhlodromips swirskii	*Tetranychus urticae*	*T*	*E, A*	Mortality; Food consumption; Fecundity	+	+	Abdel Kader et al., 2015
		Neoseiulus californicus	*Tetranychus urticae*	*T, F*	*E, N, A*	Mortality	-		Momen et al., 2014
	Mentha longifolia	*Coccinella undecimpunctata*	*Aphis punicae*	*R*	*N*	LC_{50} = 8.737 µg mL^{-1}	+		Sayed et al., 2022
	Mentha piperita	*Coccinella undecimpunctata*	*Aphis punicae*	*R*	*N*	LC_{50} = 10.334 µg mL^{-1}	+		Sayed et al., 2022
	Mentha pulegium	*Adalia bipunctata*	*Acyrthosiphon pisum, Aphis fabae, Macrosiphoniella*	*F*	*A*	LC_{50}= 0.19 µL L^{-1}	+		Kimbaris et al., 2010

		sanborni, Myzus persicae						
	Chrysoperla carnea	*Agonoscena pistaciae*	*T, F*	*E, N*	Mortality; Hatching rate	+		Azimi and Ahmadi, 2018
	Coccinella septempunctata	*Acyrthosiphon pisum, Aphis fabae, Macrosiphoniella sanborni, Myzus persicae*	*F*	*A*	LC_{50} = 0.35 µL L^{-1}	+		Kimbaris et al., 2010
	Hippodamia variegata	*Rhopalosiphum padi, Sitobion avenae, Schizaphis graminum, Metopolophium dirhodum*	*I*	*E, N*	Mortality, life history traits	-	+	Shaltoki et al., 2022
	Nesidiocoris tenuis	*Aphis gossypii, A. spiraecola, T. urticae*	*T*	*N*	Mortality	-		Papadimitriou et al., 2019
Mentha spicata	*Podisus nigrispinus*	*Alabama argillacea*	*I*	*N*	Immunohistochemical effect		-	Ático Braga et al., 2020
Mentha x piperita	*Adalia bipunctata*	*Acyrthosiphon pisum, Aphis fabae, Macrosiphoniella sanborni, Myzus persicae*	*F*	*A*	LC_{50} = 0.62 µL L^{-1}	+		Kimbaris et al., 2010
	Coccinella septempunctata	*Acyrthosiphon pisum, Aphis fabae, Macrosiphoniella sanborni, Myzus persicae*	*F*	*A*	LC_{50} = 0.67 µL L^{-1}	+		Kimbaris et al., 2010
Ocimum basilicum	*Adalia bipunctata*	*Acyrthosiphon pisum, Aphis fabae, Macrosiphoniella sanborni, Myzus persicae*	*F*	*A*	LC_{50} = 0.63 µL L^{-1}	+		Kimbaris et al., 2010
	Coccinella septempunctata	*Acyrthosiphon pisum, Aphis fabae, Macrosiphoniella sanborni, Myzus persicae*	*F*	*A*	LC_{50} = 0.58 µL L^{-1}	+		Kimbaris et al., 2010
Origanum vulgare	*Chrysoperla externa*		*T*	*N*	LD_{50} = 26,451 µg g^{-1}; Hatching rate; Fecundity	-	+	Castilhos et al., 2018
Rosmarinus officinalis	*Chrysoperla carnea*	*Agonoscena pistaciae*	*T, F*	*E, N*	Mortality, Hatching rate	+ (eggs) – (larvae)		Azimi and Ahmadi, 2018
Salvia officinalis	*Coccinella undecimpunctata*	*Aphis punicae*	*R*	*N*	LC_{50} = 6.237 µg mL^{-1}	+		Sayed et al., 2022
Salvia rosmarinus	*Coccinella undecimpunctata*	*Aphis punicae*	*R*	*N*	LC_{50} = 5.960 µg mL^{-1}	+		Sayed et al., 2022
Satureja intermedia	*Coccinella septempunctata*	*Aphis nerii*	*R*	*A*	LC_{50} = 913.722 µg mL^{-1}	-		Ebadollahi and Setzer, 2020

	Thymus vulgaris	*Chrysoperla externa*		*T*	*N*	LD_{50} = 64.493 µg g^{-1}; Hatching rate; Fecundity	-	-	Castilhos et al., 2018
Lauraceae	*Laurus nobilis*	*Phytoseiulus persimilis*	*T. urticae*	*T*	*A*	LC_{50} = 2.00×10^4 ppm; Oviposition; Food consumption; Offspring sex-ratio	-	+	Amer et al., 2016
	Laurus nobilis	*Typhlodromus negevi*	*T. urticae*	*T*	*A*	LC_{50} = 1.82×10^4 ppm; Oviposition; Food consumption; Offspring sex-ratio	-	+	Amer et al., 2016
	Litsea cubeba	*Pardosa pseudoannulata*		*F*	*A*	Orientation		-	Farid et al., 2019
Myrtaceae	*Melaleuca alternifolia*	*Podisus nigrispinus*	*Alabama argillacea*	*I*	*N*	Immunohistochemical effect		+	Ático Braga et al., 2020
Piperaceae	*Piper divaricatum*	*Neoseiulus californicus*	*Tetranychus urticae*	*R, I*	*A*	LC_{50} = 1.79 µL mL^{-1}	-		de Santana et al., 2021
Piperaceae	*Piper marginatum*	*Neoseiulus californicus*	*Tetranychus urticae*	*F*	*A*	Mortality	-		Ribeiro et al., 2016
	Piper nigrum	*Pardosa pseudoannulata*		*F*	*A*	Orientation		-	Farid et al., 2019
Poacee	*Cymbopogon citratus*	*Podisus nigrispinus*		*T*	*N, A*	Mortality; Respiratory activity; Locomotor activity	+	+	Brügger et al., 2019
Rutaceae	*Amyris balsamifera*	*Chrysoperla externa*		*T*	*N*	LD_{50} >142,657 µg g^{-1}	-		Castilhos et al., 2018
	Citrus aurantifolia	*Neoseiulus californicus*	*Tetranychus urticae*	*R, I*	*A*	LC_{50} = 0.76 µL mL^{-1}	+		de Santana et al., 2021
	Citrus limon	*Neoseiulus californicus*	*Tetranychus urticae*	*R, I*	*A*	LC_{50} = 2.26 µL mL^{-1}	-		de Santana et al., 2021
	Citrus sinensis	*Adalia bipunctata*	*Acyrthosiphon pisum, Aphis fabae, Macrosiphoniella sanborni, Myzus persicae*	*F*	*A*	LC_{50} = 1.88 µL L^{-1}	+		Kimbaris et al., 2010
		Coccinella septempunctata	*Acyrthosiphon pisum, Aphis fabae, Macrosiphoniella sanborni, Myzus persicae*	*F*	*A*	LC_{50} = 2.09 µL L^{-1}	+		Kimbaris et al., 2010
		Cryptolaemus montrouzieri	*Dactylopius opuntiae*	*R*	*A, N*	Mortality	-		El Aalaoui et al., 2019
		Neoseiulus californicus	*Tetranychus urticae*	*R, I*	*A*	LC_{50} = 3.80 µL mL^{-1}	-		de Santana et al., 2021
		Nesidiocoris tenuis	*Tuta absoluta*	*R*	*A*	Survival; Locomotor activity; Feeding activity	-	+	Soares et al., 2019
	Citrus. sinensis cv *"Pera"*	*Typhlodromus ornatus*	*Aceria guerreronis*	*R*	*A*	Mortality; Population growth	-	-	Brito et al., 2021
	Citrus spp.	*Ceraeochrysa caligata*	*Mononychellus tanajoa*	*T*	*N*	Feeding activity		+	Farias et al., 2020
	Citrus spp.	*Nesidiocoris tenuis*		*R*	*A*	Mortality; Fertility	+	+	Campolo et al., 2020
Sapotaceae	*Manilkara zapota*	*Cyrtorhinus lividipennis*	*Nilaparvata lugens*	*R*	*N, A*	Attractivity; Orientation		+	Liu et al., 2019
Siparunaceae	*Siparuna guianensis*	*Coleomegilla maculata*	*M. persicae*	*R*	*N, A*	Survival; Feeding activity	-	-	Toledo et al., 2019
		Eriopis connexa	*M. persicae*	*R*	*N, A*	Survival; Feeding activity	-	+	Toledo et al., 2019
Verbenaceae	*Lippia gracilis*	*Amblyseius largoensis*	*Raoiella indica*	*T*	*A*	Mortality	+		dos Santos et al., 2019

	Lippia microphylla	*Euborellia annulipes*	*Spodoptera frugiperda*	*T*	*N*	LD_{50} = 134.67 mg mL^{-1}	+		Alves et al., 2022
	Lippia sidoides	*Neoseiulus californicus*	*Tetranychus urticae*	*R, I*	*A*	LC_{50} = 0.78 µL mL^{-1}	-		de Santana et al., 2021
	Lippia sidoides	*Podisus nigrispinus*	*Spodoptera frugiperda*	*T*	*N*	LD_{50} = 28.43 mg g^{-1}; LT_{50}= 119 h; Locomotory activity; Repellence	+	+	Lima et al., 2020
Zingiberaceae	*Alpinia officinarum*	*Cyrtorhinus lividipennis*	*Nilaparvata lugens*	*R*	*N, A*	Attractivity; Orientation		+	Liu et al., 2019
	Curcuma longa	*Podisus maculiventris*	*Spodoptera exigua*	*T, I*	*N, A*	Mortality	+		Tavares et al., 2019

* *R* = Residual; *F* = Fumigation; *T* = Topical; *I* = Ingestion.
\# *A* = Adults; *N* = Nymphs; *E* = Eggs
§ + = significant effects; - = negligible effects

Table 2. Lethal and sub-lethal effects of essential oils (EO) toward non-target parasitoid species.

Plant family	Plant EO	Non-target species	Target pest	Exposure route *	Non-target species life stage #	Endpoint	Lethal effects §	Sub-lethal effects §	References
Amaryllidaceae	*Allium sativum*	*Trichogramma galloi*		*C*	*E*	Life history traits; transgenerational effect		+	Parreira et al., 2018
		Trichogramma pretiosum		*R*	*E, A*	Life history traits; transgenerational effect		+	Parreira et al., 2019
Anacardiaceae	*Schinus molle var. areira*	*Trissolcus basalis*	*Nezara viridula*	*F, T*	*A*	LC_{50} = 75.69 µg mL^{-1}/0.56 µg cm^{-2}; Oviposition	+	-	Werdin González et al., 2013
Apiaceae	*Carum carvi*	*Habrobracon hebetor*		*F*	*A*	LC_{50} = 0.340 µL L^{-1}; Life history traits	+	+	Razmjou et al., 2018
	Coriandrum sativum	*Cotesia glomerata*	*Plutella xylostella*	*F*	*A*	LD_{50} = 5.52 mg/filter paper	-		Yi et al., 2007
	Ferula assafoetida	*Trichogramma embryophagum Trichogramma evanescens*	*Ephestia kuehniella*	*F*	*A*	LC_{50} = 1758 ppm; Life history traits; Mating behavior	-	+	Poorjavad et al., 2014
	Foeniculum vulgare	*Habrobracon hebetor*		*F*	*A*	LC_{50} = 0.48 mL L^{-1}; Life history traits	+	+	Ahmadpour et al., 2021
	Heracleum persicum	*Habrobracon hebetor*		*F*	*A*	LC_{50} = 3.416 µL L^{-1}; Life history traits	-	+	Razmjou et al., 2018
Asteraceae	*Achillea millefolium*	*Habrobracon hebetor*		*F*	*A*	LC_{50} = 1.68 mL L^{-1}; Life history traits	-	+	Ahmadpour et al., 2021
	Artemesia vulgaris	*Cotesia glomerata*	*Plutella xylostella*	*F*	*A*	LD_{50} = 2.18 mg/filter paper	+		Yi et al., 2007
	Artemisia campestris	*Dinarmus basalis*	*Callosobruchus maculatus - Bruchus rufimanus*	*F*	*A*	Adult emergence		+	Titouhi et al., 2017
		Triaspis luteipes	*Callosobruchus maculatus - Bruchus rufimanus*	*F*	*A*	Adult emergence		+	Titouhi et al., 2017
	Artemisia herba-alba	*Dinarmus basalis*	*Callosobruchus maculatus - Bruchus rufimanus*	*F*	*A*	Adult emergence		+	Titouhi et al., 2017
		Triaspis luteipes	*Callosobruchus maculatus - Bruchus rufimanus*	*F*	*A*	Adult emergence		+	Titouhi et al., 2017
Atherospermataceae	*Laurelia sempervirens*	*Encarsia formosa*	*Trialeurodes vaporariorum*	*F*	*A*	LC_{50} = 0.86 µL L^{-1} air; LT; Fecundity	+	+	Zapata et al., 2016
Cupressaceae	*Thuja occidentalis*	*Cotesia glomerata*	*Plutella xylostella*	*F*	*A*	LD_{50} = 2.28 mg/filter paper	+		Yi et al., 2007
Lamiaceae	*Hyptis marrubioides*	*Trichogramma pretiosum,*	*Spodoptera frugiperda*	*R*	*A*	Survival; Fecundity	-	-	Bibiano et al., 2022
	Hyptis spicigera	*Dinarmus basalis*		*F*	*L, A*	Mortality; Oviposition	+	+	Sanon et al., 2010
	Hyptis suaevolens	*Dinarmus basalis*		*F*	*L, A*	Mortality; Oviposition	+	+	Sanon et al., 2010
	Lavandula angustifolia	*Cotesia glomerata*	*Plutella xylostella*	*F*	*A*	LD_{50} = 8.51 mg/filter paper	-		Yi et al., 2007

		Cotesia glomerata	*Plutella xylostella*	*F*	*A*	LC_{50} = 0.01 mg cm^{-3}	-		Yi et al., 2016
	Mentha × piperita	*Trichospilus pupivorus*	*Diaphania hyalinata*	*R*	*A*	LC_{50} = 16.09%	-		Moreira da Silva et al., 2020
	Mentha arvensis	*Pachycrepoideus vindemmiae*	*Drosophila suzukii*	*F*	*P, A*	Mortality; Adult emergence	+		Gowton et al., 2020
	Mentha piperita	*Cotesia glomerata*	*Plutella xylostella*	*F*	*A*	LD_{50} = 5.64 mg/filter paper	-		Yi et al., 2007
		Trichogramma galloi		*R*	*E*	Life history traits; transgenerational effect		-	Parreira et al., 2018
		Coccinella undecimpunctata	*Aphis punicae*	*R*	*L*	LC_{50} = 10.334 µg mL^{-1}	+		Sayed et al., 2022
		Trichogramma pretiosum		*R*	*E, A*	Life history traits; transgenerational effect		+	Parreira et al., 2019
	Mentha pulegium	*Cotesia glomerata*	*Plutella xylostella*	*F*	*A*	LD_{50} = 3.61 mg/filter paper	+		Yi et al., 2007
	Ocimum basilicum	*Dinarmus basalis*	*Callosobruchus maculatus*	*F*	*A*	LC_{50} = 0.69-1.20 µL L^{-1}; Longevity; Fecundity	+	+	Ketoh et al., 2002
		Habrobracon hebetor		*F*	*A*	LC_{50} = 0.84 mL L^{-1}; Life history traits	+	+	Ahmadpour et al., 2021
		Trichogramma pretiosum,	*Spodoptera frugiperda*	*R*	*A*	Survival; Fecundity	-	-	Bibiano et al., 2022
	Origanum vulgare	*Trichogramma galloi*		*R*	*E*	Life history traits; transgenerational effect		+	Parreira et al., 2018
		Trichogramma pretiosum		*R*	*E, A*	Life history traits; transgenerational effect		-	Parreira et al., 2019
		Trichospilus pupivorus	*Diaphania hyalinata*	*R*	*A*	LC_{50} = 2.79%	-		Moreira da Silva et al., 2020
		Trissolcus basalis	*Nezara viridula*	*F, T*	*A*	LC_{50} = 92.40 µg mL^{-1}/ 1.54 µg cm^{-2}; Oviposition	-	-	Werdin González et al., 2013
	Rosmarinus officinalis	*Cotesia glomerata*	*Plutella xylostella*	*F*	*A*	LD_{50} = 2.44 mg/filter paper	+		Yi et al., 2007
		Habrobracon hebetor		*F*	*A*	LC_{50} = 4.15 µL L^{-1}; Life history traits	+	+	Asadi et al., 2018
	Salvia officinalis	*Cotesia glomerata*	*Plutella xylostella*	*F*	*A*	LD_{50} = 2.30 mg/filter paper	+		Yi et al., 2007
		Habrobracon hebetor		*F*	*A*	LC_{50} = 18.36 µL L^{-1}; Life history traits	+	+	Asadi et al., 2018
	Thymus vulgaris	*Trichogramma galloi*		*R*	*E*	Life history traits; transgenerational effect		-	Parreira et al., 2018
		Trichogramma pretiosum		*R*	*E, A*	Life history traits; transgenerational effect		-	Parreira et al., 2019
		Trichospilus pupivorus	*Diaphania hyalinata*	*R*	*A*	LC_{50} = 10.68%	-		Moreira da Silva et al., 2020
		Trissolcus basalis	*Nezara viridula*	*F, T*	*A*	LC_{50} = 50.55 µg mL^{-1}/ 1.97 µg cm^{-2}; Oviposition	-	-	Werdin González et al., 2013
	Zataria multiflora	*Habrobracon hebetor*		*F*	*A*	LC_{50} = 1.84 mL L^{-1}; Life history traits	-	+	Ahmadpour et al., 2021
Lauraceae	*Aniba rosaeodora*	*Cotesia glomerata*	*Plutella xylostella*	*F*	*A*	LD_{50} = 7.18 mg/filter paper	-		Yi et al., 2007
	Cinnamomum camphora	*Cotesia glomerata*	*Plutella xylostella*	*F*	*A*	LD_{50} = 7.12 mg/filter paper	-		Yi et al., 2007
Meliaceae	*Carapa guianensis*	*Trichogramma galloi*		*R*	*E*	Life history traits; transgenerational effect		+	Parreira et al., 2018
		Trichogramma pretiosum		*R*	*E, A*	Life history traits; transgenerational effect		+	Parreira et al., 2019
Myrtaceae	*Corymbia citriodora*	*Psyttalia concolor*	*Ceratitis capitata*	*R*	*A*	LD_{50} = 0.04 µL/parasitoid; Oviposition; Emergence	-	-	Alves et al., 2020

	Eucalyptus camaldulensis	*Habrobracon hebetor*		*F*	*A*	LC_{50} = 1.116 µL L^{-1}; Life history traits	-	+	Razmjou et al., 2018
	Eucalyptus globulus	*Cotesia glomerata*	*Plutella xylostella*	*F*	*A*	LD_{50} = 1.59 mg/filter paper	+		Yi et al., 2007
	Eugenia uniflora	*Cleruchoides noackae*	*Thaumastocoris peregrinus*	*R*	*A, L*	Survival; transgenerational effect	+	+	Stenger et al., 2021
	Leptospermum petersonii	*Trichogramma pretiosum*	*Plutella xylostella*	*R*	*A*	Mortality; Oviposition deterrence	-	-	Purwatiningsih et al., 2012
	Melaleuca alternifolia	*Psyttalia concolor*	*Ceratitis capitata*	*R, F, I*	*A*	Mortality	-		Benelli et al., 2013
	Melaleuca viridiflora	*Cotesia glomerata*	*Plutella xylostella*	*F*	*A*	LD_{50} = 1.89 mg/filter paper	+		Yi et al., 2007
	Myrtus communis	*Cotesia glomerata*	*Plutella xylostella*	*F*	*A*	LD_{50} = 2.84 mg/filter paper	+		Yi et al., 2007
	Syzygium aromaticum	*Habrobracon hebetor*	*Galleria mellonella*	*F*	*A*	Mortality; Life history traits	+	+	Moawad et al., 2015
		Trichogramma galloi		*R*	*E*	Life history traits; transgenerational effect		+	Parreira et al., 2018
		Trichogramma pretiosum		*R*	*E, A*	Life history traits; transgenerational effect		-	Parreira et al., 2019
Pinaceae	*Cedrus atlantica*	*Psyttalia concolor*	*Ceratitis capitata*	*R*	*A*	LD_{50} = 0.04 µL/parasitoid; Oviposition; Emergence	-	-	Alves et al., 2020
Piperaceae	*Piper aduncum*	*Telenomus podisi* *Trissolcus urichi*	*Euschistus heros*	*R*	*A*	Adult emergence; Oviposition	-	-	Turchen et al., 2020
		Trichopria anastrephae	*Drosophila suzukii*	*I, T*	*A*	Mortality	-		Trombin de Souza et al., 2020
	Piper crassinervium	*Trichopria anastrephae*	*Drosophila suzukii*	*I, T*	*A*	Mortality	-		Trombin de Souza et al., 2020
	Piper gaudichaudianum	*Trichopria anastrephae*	*Drosophila suzukii*	*I, T*	*A*	Mortality	-		Trombin de Souza et al., 2020
	Piper malacophyllum	*Trichopria anastrephae*	*Drosophila suzukii*	*I, T*	*A*	Mortality	-		Trombin de Souza et al., 2020
	Piper marginatum	*Trichopria anastrephae*	*Drosophila suzukii*	*I, T*	*A*	Mortality	-		Trombin de Souza et al., 2020
	Piper nigrum	*Trichogramma galloi*		*R*	*E*	Life history traits; transgenerational effect		-	Parreira et al., 2018
		Trichogramma pretiosum		*R*	*E, A*	Life history traits; transgenerational effect		-	Parreira et al., 2019
Poaceae	*Cymbopogon citratus*	*Psyttalia concolor*	*Ceratitis capitata*	*R*	*A*	LD_{50} = 0.04 µL/parasitoid; Oviposition; Emergence	-	-	Alves et al., 2020
		Trichogramma pretiosum,		*T*	*A*	LC_{50} = 0.15%; Oviposition	-	+	Sombra et al., 2022
	Cymbopogon nardus	*Dinarmus basalis*	*Callosobruchus maculatus*	*F*	*A*	LC_{50} = 1.70-2.66 µL L^{-1}; Longevity; Fecundity	+	+	Ketoh et al., 2002
	Cymbopogon winterianus	*Trichogramma pretiosum,*		*T*	*A*	LC_{50} = 0.12%; Oviposition	-	+	Sombra et al., 2022
	Cymbopogons choenanthus	*Dinarmus basalis*	*Callosobruchus maculatus*	*F*	*A*	LC_{50} = 0.44-0.92 µL L^{-1}; Longevity; Fecundity	+	+	Ketoh et al., 2002 Ketoh et al., 2005
Rutaceae	*Agothosma betulina*	*Cotesia glomerata*	*Plutella xylostella*	*F*	*A*	LD_{50} = 7.33 mg/filter paper	-		Yi et al., 2007
	Citrus sinensis	*Trichogramma galloi*		*R*	*E*	Life history traits; transgenerational effect		+	Parreira et al., 2018
	Citrus sinensis	*Trichogramma pretiosum*		*R*	*E, A*	Life history traits; transgenerational effect		-	Parreira et al., 2019
Verbenaceae	*Aloysia citriodora*	*Trissolcus basalis*	*Nezara viridula*	*F, T*	*A*	LC_{50} = 94.23 µg mL^{-1}/ 1.53 µg cm^{-2}; Oviposition	-	-	Werdin González et al., 2013

	Lippia origanoides	*Trichogramma pretiosum,*		*T*	*A*	LC_{50} = 0.43%; Oviposition	-	+	Sombra et al., 2022
Zingiberaceae	*Zingiber officinale*	*Trichogramma galloi*		*R*	*E*	Life history traits; transgenerational effect		+	Parreira et al., 2018
		Trichogramma pretiosum		*R*	*E, A*	Life history traits; transgenerational effect		+	Parreira et al., 2019
		Trichospilus pupivorus	*Diaphania hyalinata*	*R*	*A*	LC_{50} = 8.16%	+		Moreira da Silva et al., 2020

* *R* = Residual; *F* = Fumigation; *T* = Topical; *I* = Ingestion.
\# *A* = Adults; *P* = Pupae; *L* = Larvae; *E* = Eggs
§ + = significant effects; - = negligible effects

Table 3. Percent mortality (mean ± SE) of *Eisenia fetida* earthworms after 14 days exposure to different essential oils (EO) and their toxicity toward target pests.

Plant EO	Botanical family	Target crop pest (EO toxicity)	EO dose	*E. fetida* mortality (%)	References
Deverra tortuosa	Apiaceae	*Callosobruchus maculatus* (LC_{50} = 23.1 µg cm^{-2})	200 mg kg^{-1} soil	0.0 ± 0.0 (*10 days)	Almadiy et al., 2022
Ocimum sanctum	Lamiaceae	*Spodoptera littoralis* (LD_{50}= 39.3 µg $larva^{-1}$)	500 mg kg^{-1} soil	10.0 ± 5.0	Žabka et al., 2021
Ledum palustre	Ericaceae	*Spodoptera littoralis* (LD_{50} = 117.2 µg $larva^{-1}$)	250 mg kg^{-1} soil	5.0 ± 5.0	Benelli et al., 2020a
Stevia rebaudiana	Asteraceae	*Metopolophium dirhodum* (LC^{50} = 5.1 mL L^{-1})	200 mg kg^{-1} soil	0.0 ± 0.0	Benelli et al., 2020b
Ferula assa-foetida	Apiaceae	*Spodoptera littoralis* (LD_{50} = 29.3 µg $larva^{-1}$)	200 mg kg^{-1} soil	0.0 ± 0.0	Pavela et al., 2020a
Ferula gummosa	Apiaceae	*Spodoptera littoralis* (LD_{50} = 124.4 µg $larva^{-1}$)	200 mg kg^{-1} soil	0.0 ± 0.0	
Oliveria decumbens	Apiaceae	*Spodoptera littoralis* (LD_{50} = 7.4 µg $larva^{-1}$)	200 mg kg^{-1} soil	0.0 ± 0.0	Pavela et al., 2020b
Thymus daenensis	Lamiaceae	*Spodoptera littoralis* (LD_{50} = 9.6 µg $larva^{-1}$)	200 mg kg^{-1} soil	7.5 ± 4.3	
Satureja sahendica	Lamiaceae	*Spodoptera littoralis* (LD_{50} = 23.1 µg $larva^{-1}$)	200 mg kg^{-1} soil	0.0 ± 0.0	
Satureja khuzistanica	Lamiaceae	*Spodoptera littoralis* (LD_{50} = 8.9 µg $larva^{-1}$)	200 mg kg^{-1} soil	0.0 ± 0.0	
Satureja rechingeri	Lamiaceae	*Spodoptera littoralis* (LD_{50} = 9.4 µg $larva^{-1}$)	200 mg kg^{-1} soil	0.0 ± 0.0	

Solidago canadensis	Asteraceae	*Spodoptera littoralis* (LD_{50} = 98.9 µg $larva^{-1}$)	200 mg kg^{-1} soil	0.0 ± 0.0	Benelli et al., 2019a
Solidago gigantea	Asteraceae	*Spodoptera littoralis* (LD_{50} = 84.5 µg $larva^{-1}$)	200 mg kg^{-1} soil	0.0 ± 0.0	
Ocimum gratissimum	Lamiaceae	*Spodoptera littoralis* (LD_{50} = 30.2 µg $larva^{-1}$)	200 mg kg^{-1} soil	0.0 ± 0.0	Benelli et al., 2019b
Origanum syriacum	Lamiaceae	*Spodoptera littoralis* (LD_{50} = 103.3 µg $larva^{-1}$)	200 mg kg^{-1} soil	0.0 ± 0.0	Benelli et al., 2019c
		Myzus persicae (LC_{50} = 0.005 mL L^{-1})			
Schizogyne sericea	Asteraceae	*Spodoptera littoralis* (LD_{50} > 200 µg $larva^{-1}$)	100 mg kg^{-1} soil	0.0 ± 0.0	Benelli et al., 2019d
		Myzus persicae (LC_{50} = 2.1 mL L^{-1})			
Cuminum cyminum	Apiaceae	*Spodoptera littoralis* (LD_{50} = 100.0 µg $larva^{-1}$)	100 mg kg^{-1} soil	10.0 ± 0.0	Benelli et al., 2018a
		Myzus persicae (LC_{50} = 3.2 mL L^{-1})			
Pimpinella anisum	Apiaceae	*Spodoptera littoralis* (LD_{50} = 57.3 µg $larva^{-1}$)	100 mg kg^{-1} soil	0.0 ± 0.0	
		Myzus persicae (LC_{50} = 4.3 mL L^{-1})			
Cannabis sativa	Cannabaceae	*Spodoptera littoralis* (LD_{50} = 152.3 µg $larva^{-1}$)	100 mg kg^{-1} soil	0.0 ± 0.0	Benelli et al., 2018b
		Myzus persicae (LC_{50} = 3.5 mL L^{-1})			

Foeniculum vulgare	Apiaceae	*Myzus persicae* (LC_{50} = 0.6 mL L^{-1})	240.7 mg kg^{-1} soil	12.5 ± 5.0	Pavela, 2018