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

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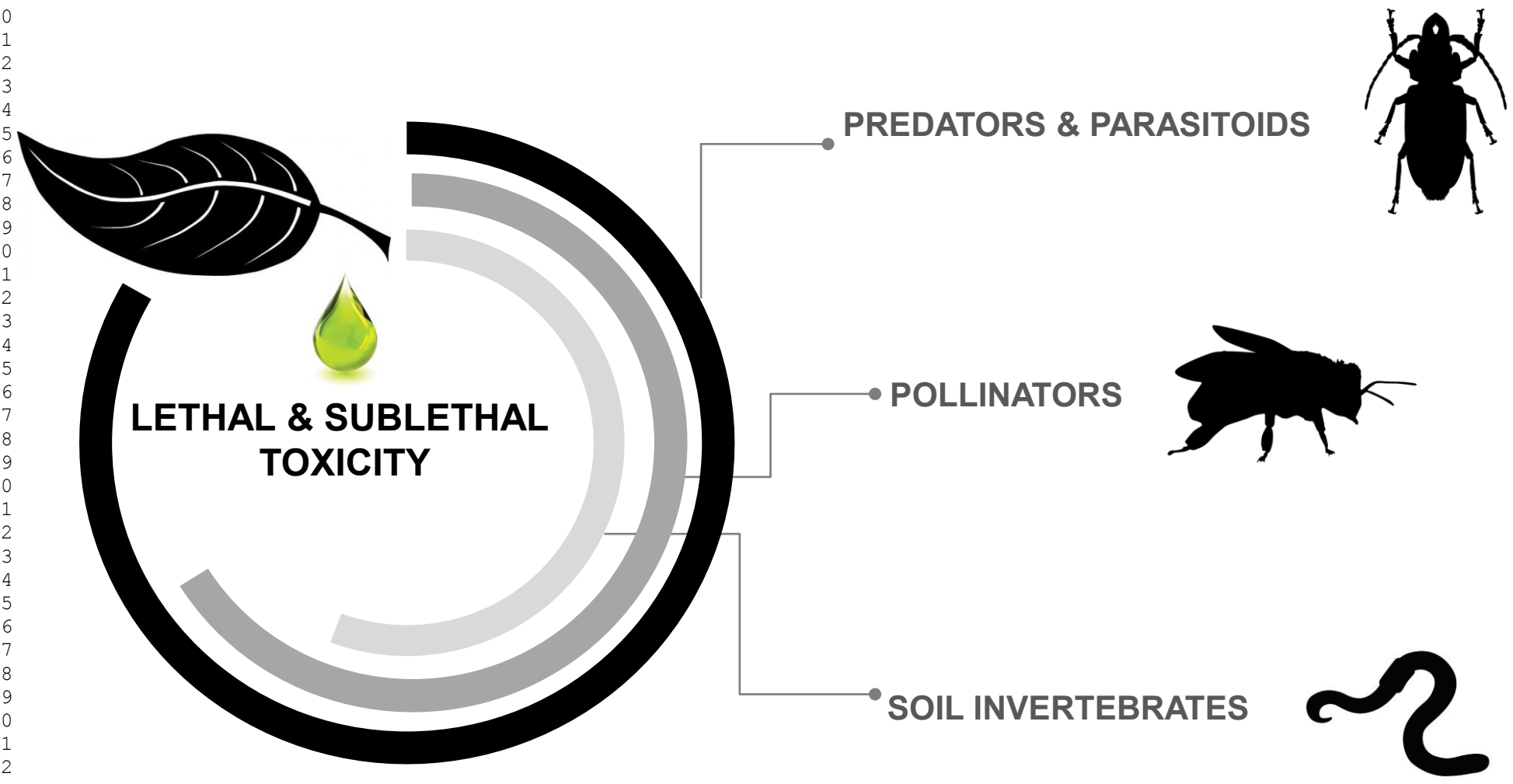
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Highlights

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- 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

ESSENTIAL OIL-BASED INSECTICIDES AND ACARICIDES



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1 *Invited Review for Biological Control*

2 2

3 3 **Non-target effects of essential oil-based biopesticides for crop protection: impact on natural**
4 **enemies, pollinators, and soil invertebrates**

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27 **Abstract**

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

45
46 **Key words:** biocontrol; Integrated Pest Management; lethal effects; sublethal effects; parasitic wasp;
47 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

76 pest control, essential oils (EOs) are promising active ingredients for insecticidal formulations due to
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277 their worldwide availability and relative low cost and their presumed safety for human health and the
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478 environment ([Isman, 2020](#); [Li et al., 2022](#); [Palermo et al., 2021](#)). EOs are secondary metabolites
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779 produced by plants for a variety of purposes and they are involved in indirect plant defense
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980 mechanisms (i.e., against both biological and abiotic stress), and play a key role in signaling
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1281 processes, including plant attractiveness toward beneficials and pollinators. EOs are produced by
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1482 several plant species, i.e. the so-called aromatic plants belonging to a panel of botanical families such
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1783 as Asteraceae, Apiaceae, Lamiaceae, Lauraceae, Myrtaceae, Verbenaceae, Geraniaceae,
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1984 Zingiberaceae, Pinaceae, and others ([Benelli et al., 2017](#); [Pavela et al., 2021a, 2021b](#); [Spinozzi et al.,](#)
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2185 [2021](#)). They are synthesized and eventually stored in secretory structures of epidermal or
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2486 parenchymatic origin which are distributed in different plant parts or organs, such as roots, bark,
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2687 leaves, seeds, fruits, bark, and tubers. Furthermore, EOs produced from the same plant but extracted
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2988 from different organs can vary significantly both in terms of chemical composition and yield. Even
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3189 when the same plant species is considered, the yield and composition of EOs may vary with the
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3490 cultivated environment and the plant genetic background leading to the presence of different
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3691 chemotypes within the same species ([Pavela and Benelli, 2016](#)).

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3992 EOs are phytocomplexes composed of a blend of substances (i.e., often more than 50),
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4193 including terpenes (monoterpenoids and sesquiterpenoids), the most frequent constituents, but also
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4494 aromatic (i.e., phenylpropanoids, aldehydes, alcohols, esters, etc.) and aliphatic compounds (i.e.,
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4695 alkanes, aldehydes, alcohols, ketones, esters) and others (i.e., polyacetylenes). It is quite common that
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4896 a single compound accounts for more than 20% of a given EO; as an example, the relative content of
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5197 D-limonene in orange EO exceeds 50% of total components ([Buriani et al., 2020](#)).

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5398 Because EOs are accumulated inside plant organs, they must be collected from plant tissues
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5699 using different extraction techniques. The most common extraction techniques are hydrodistillation
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59100 (HD), steam distillation, and cold pressing (CP). These sometimes are characterized by a variety of
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61101 disadvantages, including low efficiency and yield, and degradation of some molecules ([Reyes-Jurado](#)

102 et al., 2014). Due to the above-mentioned limitations, several new techniques have been developed
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103 to effectively extract EOs, such as microwave-assisted extraction (MAE), which improves the
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104 production efficiency while reducing time and energy consumption during the process (Sawamura,
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105 2011; Fiorini et al., 2020).

106 The EO characteristics (i.e., high volatility and biodegradability, low persistence in the
10 environment) which make these phytocomplexes promising active ingredients for biopesticides, also
11
107 environment) which make these phytocomplexes promising active ingredients for biopesticides, also
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13
108 limit their application as commercial plant protection products. These limitations reduce the
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109 possibility of the use of EOs as such and the difficulties in patenting the EO-based pesticide
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110 formulations have limited the spread of commercial formulations. Since these bioinsecticides are
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22 moderately commercialized and represent a restricted market at present, it can be assumed that EOs
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24 are unsuccessful control tools under field conditions. Nonetheless, field evaluations showed that EOs
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113 can be effective in some situations and can obtain pest control levels comparable to organo-synthetic
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114 pesticides (Isman et al., 2011; Smith et al., 2018). Despite the huge number of studies about EO
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115 bioactivity against pests, the main commercialization of bioinsecticides based on EOs dates over a
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116 decade ago in the USA and just 6-7 years in the EU (Isman, 2020). Commercially available
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117 formulations may contain a single EO or EO constituent, a mixture of different EOs, as well as a
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118 blend of synthetically produced terpenoids. These formulations and EOs in general are often
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41
119 acknowledged to be safe for the environment and human health based on the physicochemical
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120 properties of these compounds derived from their respective structures; however, few studies,
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121 compared to the large bibliography available about EOs toxicology against pest species, focused on
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122 the ecotoxicological impact of EOs and EO constituents against non-target species (Haddi et al. 2020;
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123 Turchen et al. 2020).

124 In a recent paper, Ferraz et al. (2022) reviewed the impact of both EOs and plant extracts on
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125 non-target organisms, namely microalgae, crustaceans, fishes, plants, and soil (micro)organisms;
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126 however, terrestrial invertebrate species, such as BCA and pollinators, were not considered. Natural
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127 enemies of crop pests, as well as pollinators, can directly contact with pesticides on sprayed crops
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128 and contaminated nearby vegetation, and they can feed on treated plants/preys/hosts. Furthermore,
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129 soils can also be polluted by residues of pesticide applications due to drift phenomena, and the
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130 abundance and variety of invertebrate species in soils is a recognized bioindicator for environmental
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131 health and pollution, which should be accounted in agroecosystems (Burger, 2007). In this context,
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132 this review focuses on the main findings about lethal and sublethal effects of EOs against non-target
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133 terrestrial invertebrates in agriculture, including beneficial arthropods (i.e., predators, parasitoids,
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134 pollinators), as well as soil non-target species.
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17 18 19 **2. Invertebrate predators of crop arthropod pest species** 20

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22 Among beneficials playing a key role in biological control programs against several pests, predators
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24 are valuable control agents due to their ability to feed on and kill several to many individual prey
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26 during their lifetimes. Predatory beetles, flies, lacewings, true bugs, and predatory mites are just some
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28 examples of predators used in biological control programs. The side effects of EOs on these predators
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30 are quite variable depending on different parameters, including plant species, EO formulation,
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32 application technique, and non-target species and life stage (Table 1).
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35 36 37 38 39 *2.1. Lethal effects of EOs toward invertebrate predators* 40

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42 EOs are generally considered safe for non-target predators, because of their high mobility and their
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44 larger size compared to target species. In this regard, it is commonly acknowledged that higher doses
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46 of toxicants are needed to kill larger species or specimens, although there are exceptions. In contact
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48 toxicity tests on *Aphis punicae* Passerini (Hemiptera: Aphididae) adults, it was shown that LC_{50s} for
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50 various EOs were approximately four-fold lower than those estimated for *Coccinella*
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52 *undecimpunctata* L. (Coleoptera: Coccinellidae) larvae (Sayed et al., 2022). Furthermore, *Satureja*
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54 *intermedia* C. A. Mey EO is a good candidate to develop plant-derived aphicides because of its
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56 toxicity against *Aphis nerii* Boyer de Fonscolombe (Hemiptera: Aphididae), coupled with its relative
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58 safety to the generalist predator *Coccinella septempunctata* L. (Coleoptera: Coccinellidae)
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154 (Ebadollahi and Setzer, 2020). On the other hand, fumigation with four EOs toxic to aphid pests
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155 (*Mentha pulegium* L., *Mentha x piperita* L., *Ocimum basilicum* L., and *Citrus sinensis* (L.) Osbeck
3
156 EOs) caused variable mortality on two coccinellid predator species, the seven-spotted ladybird *C.*
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157 *septempunctata* and the two-spotted ladybird *Adalia bipunctata* L. (Coleoptera: Coccinellidae) with
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158 distinctive selective toxicity ratios depending on the considered aphid species, coccinellid predator
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159 and EO (Kimbaris et al., 2010).
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160 Bioactive botanical compounds can be more selective than commercial synthetic insecticides
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161 (Benelli et al., 2019c, 2018a; Pavela, 2018); as an example, the EO of *Lippia sidoides* Cham.,
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162 (Verbenaceae) and its major compound thymol were less toxic than deltamethrin toward the predator
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163 *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae), a predator of *Spodoptera frugiperda* Smith
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164 (Lepidoptera: Noctuidae); besides, deltamethrin led to quicker mortality to nymphs of *P. nigrispinus*
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165 (LT₅₀= 0.36 h) compared to EO (LT₅₀= 119 h) and thymol (LT₅₀= 93 h). Moreover, these botanical
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166 compounds acted against the pest faster than the synthetic insecticide (Lima et al., 2020). Similarly,
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167 dichlorvos was more toxic (LD₅₀ 9.0 × 10⁻¹⁰ mg cm⁻³) against *Orius strigicollis* Poppius (Hemiptera:
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168 Anthocoridae), compared with *O. basilicum* EO constituents, whose LD₅₀ values ranged from 0.0127
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169 to > 0.23 mg cm⁻³ (Kim et al., 2015).
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170 Nevertheless, EO-based formulations are not always selective to predators of target species.
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171 As an example, LC₅₀ values for *Vanillosmopsis arborea* Baker and *Lippia microphylla* Cham. EOs
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172 topically applied to *S. frugiperda* larvae were 172.86 mg mL⁻¹ and 104.52 mg mL⁻¹ respectively, but
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173 the lethal concentrations for the generalist predator *Euborellia annulipes* Lucas (Dermaptera:
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174 Anisolabididae), were similar or even lower (*V. arborea* LC₅₀ = 160.2 mg mL⁻¹; *L. microphylla* LC₅₀
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175 = 134.67 mg mL⁻¹) (Alves et al., 2022). Furthermore, EOs can cause mortality of predators both by
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176 direct contact, as well as by ingestion of treated prey, as supported by the survival of *Podisus*
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177 *maculiventris* Say (Heteroptera: Pentatomidae) to *Curcuma longa* L. EO and its major components
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178 after topical application and ingestion of treated *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae)
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179 larvae (Tavares et al., 2019). In some cases, EOs can be safe to adults and pre-imaginal stages of
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180 predators while causing mortality of eggs, impairing egg hatching; this is the case of *Rosmarinus*
181 *officinalis* L. EO, which caused low mortality rate toward *Chrysoperla carnea* Stephens (Neuroptera:
182 Chrysopidae) larvae, however having negative effects on the eggs hatching rate of the same species
183 (Azimi Zadeh and Ahmadi, 2018).

184 The use of EOs as acaricides has also been studied in depth, since phytophagous mites are
185 serious pests in greenhouse and field agricultural ecosystems. Among the EOs used for the control of
186 mites, the one extracted from *Lippia sidoides* Cham. exhibited a good toxicity against *Tetranychus*
187 *urticae* C.L. Koch (Acari: Tetranychidae) as well as a good selectivity towards the predator mite
188 *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) (de Santana et al., 2021). This predatory
189 mite was also more tolerant than the target pest to *Melissa officinalis* L. (Momen et al., 2014), *Piper*
190 *aduncum* L., *Melaleuca leucadendra* L., and *Schinus terebinthifolius* Raddi EOs, as well as their
191 binary blends (de Araújo et al., 2020), while it was sensitive to *P. marginatum* Jacq. EO (Ribeiro et
192 al., 2016). Similar results were reported for *Typhlodromus ornatus* Denmark & Muma (Acari:
193 Phytoseiidae), a naturally occurring generalist predatory mite in coconut plantations, which was not
194 affected by sweet orange (*C. sinensis*) cv “Pera” EO at the lethal concentrations used against the
195 target mite species *Aceria guerreronis* Keifer (Acari: Eriophyidae) (Brito et al., 2021). The selectivity
196 of the tested EOs towards *T. ornatus* may be related to the biological, anatomical, and physiological
197 differences between predators and their prey, such as the integument or presence of detoxifying
198 enzymes (Sato et al., 2006; Tsolakis and Ragusa, 2008). Conversely, dos Santos et al. (2019) reported
199 that the EO from *Lippia gracilis* Schauer was toxic both against the target species *Raoiella indica*
200 Hirst (Acari: Tenuipalpidae) as well as against the predator mite *Amblyseius largoensis* (Muma)
201 (Acari: Phytoseiidae), since the LC₅₀ (4.99 mg/mL) of the EO estimated for *R. indica* caused
202 48.33 ± 3.07% mortality to *A. largoensis*.

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

206 chemotypes (i.e., major constituent pulegone or piperitone) revealed a good insecticidal activity
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207 against *Aphis gossypii* Glover (Hemiptera: Aphididae), *A. spiraeicola* Patch (Hemiptera: Aphididae)
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208 and *T. urticae* (Acari: Tetranychidae) at 1000 μ L/L of EO concentration in spray applications
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209 irrespective of the chemotype; the impact of both EOs on the polyphagous predator *Nesidiocoris*
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210 *tenuis* (Reuter) (Hemiptera: Miridae) was negligible (Papadimitriou et al., 2019). Similar results were
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211 highlighted by Ricupero et al., (2022) in which garlic EO based nano-emulsion revealed a significant
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212 toxicity against *Tuta absoluta* while no lethal effects were highlighted towards *N. tenuis* adults. On
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213 the other hands, the same formulation had a significant impact on the progeny produced by females
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214 allowed to develop on treated plants. Shaltoki et al. (2022) confirmed the negative effect of
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215 pennyroyal EO applications towards *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae)
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24
216 eggs and first-instar larvae, affecting both survival and reproductive performances of the developed
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217 adult beetles.

218 Considering different closely related species, the evaluation of different *Citrus* peel EOs
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219 towards the generalist predator *N. tenuis* demonstrated a significant variability in terms of acute
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220 mortality and side-effects depending on the type of formulation, the EO used and the different residual
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221 times (Campolo et al., 2020). Moreover, exposure time is also a key factor determining the effects of
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222 insecticides on non-target species; indeed, the EOs extracted from *Artemisia sieberi* Besser,
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223 *Pelargonium graveolens* L'Hér., and *Ferula gummosa* Boiss. Showed similar toxicity against the pest
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224 *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Zandi-Sohani et al., 2018). Conversely, their
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225 effects on the generalist predator *Orius albidipennis* (Rueter) (Hemiptera: Anthocoridae) varied
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226 according to the EO and the exposure time, although the LC₅₀ values against predators were
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227 significantly higher than those of target pest species (Zandi-Sohani et al., 2018). These results suggest
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228 that the compatibility of EO-based pesticides in organic agriculture can be improved through careful
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229 timing of treatment and release of natural enemies. Indeed, most of these substances exert their toxic
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230 activity only at high doses for a limited period after treatment and, in general, the toxicity towards
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231 natural enemies is significantly reduced with the aging of residues both toward generalist and specific
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232 predators (Brito et al., 2021; Campolo et al., 2020, 2017). Although low persistence is a desirable
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233 trait in conventional pest management, the rapid degradation and volatility of EOs in the
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234 agroecosystems can limit their effectiveness against the target species and, at the same time, can be
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235 useful where natural enemies need to be protected.

236 The formulation of EO in organic solvent can also mitigate potential negative effects toward
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11 non-targets, while maintaining pesticidal activity (Abdel Kader et al., 2015); as an example, *Varronia*
1237 *curassavica* Jacq. (Boraginaceae) oil-in-water emulsion revealed a good insecticidal activity against
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238 the target pests *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and *T. urticae*, while it did not affect
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239 the survival of the generalist predator *Ceraeochrysa cubana* Hagen (Neuroptera: Chrysopidae), even
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240 when applied at the highest tested application rate (1%) (Andrade et al., 2021). Amer et al. (2016)
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241 evaluated the toxicity of *Laurus nobilis* L. EO and its commercial formulation Lauricide® on the
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242 predatory mite, *Typhlodromus negevi* Swirski and Amitai and *Phytoseiulus persimilis* Athias- Henriot
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243 (Acari: Phytoseiidae); predatory females were found to be more tolerant than *T. urticae* females to
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244 both materials, with LC₅₀ values higher for the EOs (1.82×10^4 and 2.00×10^4 ppm for *T. negevi* and
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245 *P. persimilis*, respectively) compared to the formulation (0.28×10^4 and 0.40×10^4 ppm).

246 The evaluation of the efficacy of various conventional and biological pesticides against the
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247 prickly pear cactus cochineal *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) and their
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248 selectivity towards its natural predator *Cryptolaemus montrouzieri* Mulsant (Coleoptera:
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249 Coccinellidae) have been investigated by El Aalaoui et al. (2019). Among the tested insecticides, the
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46
250 Prev-am® commercial formulation based on d-limonene (the main compound of sweet orange EO)
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48
251 was effective in controlling *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) and, at the
49
50
252 same time, showed a low impact on adults and larvae of the coccinellid predator (El Aalaoui et al.,
51
52
253 2019). Similarly, Soares et al. (2019) demonstrated that the survival of the mirid predator *N. tenuis* is
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54
254 not affected by Prev-am® at various concentrations.

255 The susceptibility of predator species to EOs may be caused by physiological alterations. The
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57
256 EOs from *Mentha spicata* L. and *Melaleuca alternifolia* (Maiden & Betche) Cheel were used to
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257

258 evaluate the effect of ingestion of treated prey by *P. nigrispinus*. [Ático Braga et al. \(2020\)](#)
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259 demonstrated that *M. alternifolia* EO administration caused an elongation of digestive cells, followed
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260 by cell lysis and tissue necrosis, while *M. spicata* caused just a reduction in the carbohydrate levels.
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261 8 9 262 2.2. Sublethal effects of EOs toward invertebrate predators

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263 Apart from acute lethal toxicity, EO administration can influence various life-history traits of non-
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264 target predators such as their reproductive performance and predatory ability, which are the most
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265 investigated biological parameters in this context and are designated sublethal effects (see Desneux
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19
266 et al., 2007 for a thorough review). EOs and their terpenoid constituents can affect the physiology of
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267 insects and mites in different ways and places, resulting in a disruption of reproductive processes
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24
268 such as oogenesis, vitellogenesis, maturation, and spermatocyte growth ([Shaltoki et al., 2022](#)). The
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26
269 LC₅₀ and LC₈₀ of the ‘Pera’ sweet orange EO, estimated for *A. guerreronis*, did not affect the
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28
270 population growth of the generalist predatory mite *T. ornatus* ([Brito et al., 2021](#)). In contrast, sublethal
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31
271 effects on fecundity and fertility were observed in *Chrysoperla externa* (Hagen) (Neuroptera:
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33
272 Chrysopidae) for d-limonene, while oregano EO affected only the fecundity of this green lacewing
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273 ([Castilhos et al., 2018](#)).

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274 Predatory behavior can also be influenced by insecticidal and acaricidal treatments; the
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275 walking activity of *N. tenuis* adults exposed to leaves treated with synthetic pyrethroid (lambda-
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43
276 cyhalothrin) and *Citrus* EO-based biopesticide (Prev-am[®]) was significantly higher compared to the
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46
277 control treatment, while the predatory voracity was reduced by lambda-cyhalothrin and increased by
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48
278 Prev-am[®] treatment (Soares et al. 2019). Similarly, Passos et al., (2022) demonstrated that *N. tenuis*
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279 adults biological traits (fertility and orientation behaviour) were negatively affected by exposing the
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280 mirid to the tested EOs formulations (garlic, anise, fennel and lavender).

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281 In contrast, [Abdel Kader et al. \(2015\)](#) evaluated the effect of *M. officinalis* EO and its
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58
282 commercial formulation (Melissacide[®]) against females of two predatory phytoseid mites,
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60
283 *Typhlodromips swirskii* (Athias Henriot) (Acari: Phytoseiidae), and *Neoseiulus barkeri* (Hughes)

284 (Acari: Phytoseiidae), showing that Melissacide[®] can reduce food consumption, while moderate
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285 effects were highlighted in the daily number of deposited eggs. Similarly, eggs of both predatory
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4
286 mites were not influenced by *L. nobilis* EO, while its formulation reduced oviposition and food
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287 consumption, also influencing the sex-ratio of the offspring (Amer et al., 2016). The effect of an EO
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288 can be species-specific; the exposure to *Siparuna guianensis* Aubl. EO did not affect the predatory
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11
289 abilities of *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae) but increased the abilities
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13
290 of *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) to prey upon *M. persicae* (Toledo et al.,
14
15
291 2019). Similarly, *Ceraeochrysa caligata* B. (Neuroptera: Chrysopidae) larvae surviving exposure to
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17
292 *Citrus* EO exhibited higher predatory ability when faced with prey scarcity (Farias et al., 2020).
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19
20
293 Brügger et al. (2019) investigated the impact of lemongrass EO and its constituents against *P.*
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23
294 *nigrispinus*; the terpenoid constituents of lemongrass EO had a negative effect on respiration rate of
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25
295 the hemipteran predator, probably due to muscle paralysis, disruption of oxidative phosphorylation
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296 processes and dysregulation of the breathing activities, which could explain the reduced predatory
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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

310 *Pardosa pseudoannulata* Boesenberg and Strand (Araneae: Lycosidae) was evaluated in choice tests
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311 using EOs of *Piper nigrum* L. and *Litsea cubeba* (Lour.) Pers., or their mixture as given cues,
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312 revealing that these EOs had no significant influence on the orientation of the predator while the
6
313 mixture elicited its attraction (Farid et al., 2019).
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3. Parasitoids of crop insect pest species

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316 Parasitoids represent one of the best weapons among the BCA used against various pests. Their
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317 success is due to their effectiveness in intercepting the host, which is generally more sophisticated
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323 2018). But is it real? The effects of EOs toward parasitoids are summarized in **Table 2**.

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3.1. Lethal effects of EOs toward parasitoids

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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:

336 Trichogrammatidae), so they potentially could be used in *S. frugiperda* integrated pest management
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337 programs (Bibiano et al., 2022). Similarly, oregano, peppermint, and thyme EOs were more toxic to
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338 different instars of *Diaphania hyalinata* (L.) (Lepidoptera: Pyralidae) than toward its adult parasitoid,
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339 *Trichospilus pupivorus* Ferrière (Hymenoptera: Eulophidae), in residual contact toxicity trials,
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340 whereas the toxicity of ginger EO was comparable for both the pest and the natural enemy (Moreira
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341 Da Silva et al., 2020). Therefore, EOs are not always harmless for parasitoids, as reported by Zapata
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14
342 et al. (2016), who evaluated the toxicity of *Laurelia sempervirens* (Ruiz & Pav.) Tul.
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16
343 (Atherospermataceae) EO against adult *Trialeurodes vaporariorum* (Westwood) (Hemiptera:
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19
344 Aleyrodidae) ($LC_{50} = 3.77 \mu\text{L L}^{-1}$ air) and the parasitoid *Encarsia formosa* (Gahan) (Hymenoptera:
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345 Aphelinidae) ($LC_{50} = 0.86 \mu\text{L L}^{-1}$ air).

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

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352 Plant species and EO chemical characteristics deeply influence the toxicity toward parasitoids;
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353 *Habrobracon hebetor* Say (Hymenoptera: Braconidae), natural enemy of several Lepidoptera, was
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354 susceptible to *Foeniculum vulgare* Mill. ($LC_{50}=0.48 \mu\text{L L}^{-1}$) and *O. basilicum* EOs ($LC_{50}=0.84 \mu\text{L L}^{-1}$),
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355 while *Achillea millefolium* L. ($LC_{50}=1.68 \mu\text{L L}^{-1}$) and *Zataria multiflora* Boiss EOs ($LC_{50}=1.04$
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356 $\mu\text{L L}^{-1}$) were less toxic (Ahmadpour et al., 2021). Furthermore, LC_{50} values for *R. officinalis* and
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357 *Salvia officinalis* L. EOs against this braconid species are 4.15 and $18.36 \mu\text{L L}^{-1}$ of air, respectively.
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358 In addition, EOs extracted from five species of the genus *Piper* were tested against the pupal
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56
359 parasitoid *Trichopria anastrephae* Lima (Hymenoptera: Diapriidae), natural enemy of *D. suzukii*, but
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58
360 these EOs caused low parasitoid mortality (< 20%) both through ingestion and topical application
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61
361 (Trombin de Souza et al., 2020).

362 Different administration techniques can determine various degrees of selectivity. As an
1
363 example, the application of different EOs as fumigants towards adults of the egg parasitoid *Trissolcus*
3
364 *basalis* Wollaston (Hymenoptera: Scelionidae) highlighted a good selectivity of the tested EOs, while
6
365 the same EOs were not selective in residual contact toxicity trials (Werdirn González et al., 2013).
8
366 Three EOs [*Lippia origanoides* Kunth, *Cymbopogon winterianus* Jowitt ex Bor, *Cymbopogon*
10
367 *citratius* (DC.) Stapf] showed selectivity for the parasitoid *T. pretiosum* in residual contact toxicity
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368 experiments, resulting in a LC₅₀ of 0.43%, 0.15% and 0.12% for *L. origanoides*, *C. citratius* and *C.*
14
369 *winterianus*, respectively (Sombra et al., 2022). Time interval between EO treatment and parasitoid
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16
369 release can be a key factor for EO selectivity. The parasitoid wasps *Dinarmus basalis* (Rond.)
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370 (Hymenoptera: Pteromalidae) and *Triaspis luteipes* (Thomson) (Hymenoptera: Braconidae),
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371 developing on bruchid host larvae, were tested for their susceptibility to *Artemisia herba-alba* Turra
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24
372 and *A. campestris* L. EOs. *Dinarmus basalis* was susceptible in fumigation trials while parasitoids
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373 released 6 days after treatment had reduced negative effects, as well as *T. luteipes*, whose emergence
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374 was just slightly reduced (Titouhi et al., 2017). Similarly, Ketoh et al. (2005, 2002) demonstrated that
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375 EO vapors and residues (6 days from treatment) can be highly toxic toward adult *D. basalis*, and all
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32
376 developmental stages of this parasitoids were very susceptible to *Hyptis spicigera* Lam and *H.*
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34
377 *suaveolens* (L.) Kuntze EOs (Sanon et al., 2011).
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379 Parasitoid instars can be differentially affected by EO administration according to their
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380 biology (i.e., larval-pupal/egg and endo/ecto-parasitoids) because they can be protected by the
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43
381 parasitized host. Adults of *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae) a
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45
382 pupal parasitoid of *D. suzukii*, were susceptible to EO vapors extracted from *Mentha arvensis* L.,
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383 whereas the immature instars were unaffected by this EO, probably because they are developing
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384 within the host puparia which can protect the parasitoid from toxicants (Gowton et al., 2020).
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55 Similar to pupal cases, the egg chorion may protect parasitoids from the negative impacts of
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58 EOs targeting their host pests. Indeed, *P. aduncum* EO applied against *Euschistus heros* (F.)
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60
387 (Hemiptera: Pentatomidae), a key soybean pest, did not affect the emergence of either *Telenomus*
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388 *podisi* (Ashmead) (Hymenoptera: Platygasteridae) and *Trissolcus urichi* (Crawford) (Hymenoptera:
1
389 Platygasteridae) egg parasitoids (Turchen et al., 2016). Conversely, the preimaginal stages of
3
390 *Trichogramma embryophagum* (Hartig) (Hymenoptera: Trichogrammatidae) and *Trichogramma*
4
591 *evanescens* Westwood (Hymenoptera: Trichogrammatidae), developing inside *Ephestia kuehniella*
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392 (Zeller) (Lepidoptera: Pyralidae) eggs, suffered reduced emergence rate due to the application of
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1393 *Ferula assafoetida* L. EO (Poorjavad et al., 2014). Several EO compounds can penetrate the egg
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404 ([Parreira et al., 2019](#)).

405 Most of the EOs-based insecticides or acaricides are formulated by using single compounds
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406 or oils, even though mixtures of different EOs, or compounds, can improve their efficacy against
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413 more tolerant than the medfly, as the LD₅₀ value estimated for *P. concolor* was 6.5-fold higher than

414 *C. capitata* one (Alves et al., 2020). Similar results were reported by Benelli et al. (2013) who found
1
415 that *M. alternifolia* EO was more toxic to *C. capitata* than to its parasitoid *P. concolor* in contact,
3
416 fumigation, and ingestion toxicity trials.
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418 3.2. Sublethal effects of EOs toward parasitoids

419 Compared to predator species, more studies present results about sublethal effects of EO on
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420 the life-history traits of parasitoids, mainly focusing on the parasitization ability of the adult females.
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421 Parreira et al. (2019) identified two EOs (*Allium sativum* and *Carapa guianensis*) decreasing the
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18
422 parasitism rate of *T. pretiosum* females (33 and 70%, respectively), indicating these EOs as slightly
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423 harmful (class 2) in relation to parasitism according to IOBC toxicity categories. Furthermore,
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22
424 *Leptospermum petersonii* F.M. Bailey EO appeared harmless to *T. pretiosum*, since both the
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24
425 oviposition rate and the adult survival were not affected by the EO treatments (Purwatiningsih et al.,
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26
426 2012). In contrast, the closely-related species *T. galloi* reduced its parasitization ability (between 30
27
28
427 to 79%) in F₁ and F₂ parasitoid generations after treatments with *A. sativum*, *C. guianensis*, *C.*
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30
428 *sinensis*, *Azadirachta indica* A. Juss. and *O. vulgare* EOs (Parreira et al., 2018). Nevertheless, the EO
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429 from *Z. officinale* completely nullified the parasitism rate of *T. pretiosum* on eggs of *E. kuehniella*,
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430 suggesting a strong repellent activity of this EO toward the parasitoid females (Parreira et al., 2019).
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431 Nevertheless, some EOs have no effect on the parasitism rate of parasitoid species; as an
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432 example, EOs from *O. vulgare* and *Thymus vulgaris* L. were selective fumigants, evoking no change
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45
433 in parasitoid behavior, and one week-old residues were safe also to *T. basalis* adults (Werding
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47
434 González et al., 2013). Similarly, *P. concolor* treated with a mixture of EOs at 1.8% presented no
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435 deleterious effects on the percentage of parasitized *C. capitata* larvae, whereas parasitism rate
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436 decreased during the 2 first days after treatment at the highest concentration tested (4.8%) (Alves et
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54
437 al., 2020). Furthermore, the differences of acute toxicity among EOs do not always correspond to
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56
438 their side-effects (Sombra et al., 2022); *A. millefolium* and *Z. multiflora* EOs had lower LC₅₀ values
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439 on parasitoid wasps *H. hebetor* than *F. vulgare* and *O. basilicum* EOs, although LC₃₀ values affected
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440 the fecundity and fertility of treated wasps similarly for all the tested EOs (Ahmadpour et al., 2021).
3

441 The reproductive ability of *E. formosa* was significantly affected by the administration of low
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442 doses (i.e., lower than LC₅₀ for target pests) of *L. sempervirens* EO, but this treatment also decreased
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9
443 the host parasitism ability and the total number of offspring produced by each parasitoid female
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11
444 ([Zapata et al., 2016](#)). *Zingiber officinale* EO was able to reduce the *T. galloi* offspring production of
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14
445 F₁ and F₂ generations between 30 and 99%, showing a transgenerational effect, while this EO had
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16
446 little influence on the female parasitism rate (Parreira et al., 2018). Nevertheless, the sex ratios of the
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19
447 two *T. galloi* generations were neither affected in *T. galloi* nor in *T. pretiosum* ([Parreira et al., 2019,](#)
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21
448 [2018](#)). Under laboratory conditions, *Eugenia uniflora* L. EO was effective against different life stages
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24
449 of *Thaumastocoris peregrinus* (Carpintero & Dellapé) (Hemiptera: Thaumastocoridae), but this EO
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26
450 was harmful towards the egg parasitoid *Cleruchoides noackae* Lin & Huber (Hymenoptera,
27
28
451 Mymaridae), having also transgenerational effects ([Stenger et al., 2021](#)). The fertility life table
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31
452 parameters of *Trichogramma embryophagum* (Hartig) (Hymenoptera: Tricogrammatidae) and
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33
453 *Trichogramma evanescens* Westwood (Hymenoptera: Tricogrammatidae) were assessed after
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36
454 treatments with *F. assafoetida* EO, and female longevity, total number of offspring, number of female
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38
455 offspring per female (sex ratio), progeny wing abnormality in the progeny and developmental time
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41
456 were negatively altered for both species when parasitoid females were treated with very low EO
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43
457 concentrations (i.e., LC₀₁) ([Poorjavad et al., 2014](#)). Furthermore, the same research also investigated
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45
458 the reproductive behavior of *Trichogramma* spp., which can influence the parasitoid performances.
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459 [Poorjavad et al. \(2014\)](#) noted that mating success and occurrence were affected by EO, as well as the
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460 duration of copula were reduced; on the other hand, the time spent by males in mating searching
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461 behavior increased, highlighting some impairments caused by EO administration.
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462 Apart from reproductive impairments, other side-effects can involve the developmental time
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463 of both treated parasitoid and their offspring. *Dinarmus basalis* females almost halved the parasitism
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464 rate on bruchid larvae treated with *Hyptis* spp. EOs, and the eclosed larvae presented a significantly
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465 extended pre-imaginal developmental time (Sanon et al., 2011). Some EOs can thus influence
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466 population dynamic parameters such as: population growth rate (r or λ), net reproductive rate (R_0)
3
467 and gross reproductive rate (GRR) of parasitoid species (Ahmadpour et al., 2021; Razmjou et al.,
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6
468 2018). As an example, Asadi et al. (2018) reported that the EOs of *R. officinalis* and *S. officinalis* can
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9
469 negatively affect several parameters of the parasitoid *H. hebetor*, including adult longevity, fecundity
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11
470 and fertility, population growth rate, gross and net reproductive rates, mean generation and doubling
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13
471 time, survival and death rate and cohort survival rate. Besides, also adult longevity can be reduced;
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15
472 fumigation with clove EO and geranial ($0.5\mu\text{L } 50\text{mL}^{-1}$ of air) caused above 90% reduction in egg
16
17
473 hatchability and life span of *H. hebetor* adults (Moawad et al., 2015). Similarly, the longevity of *T.*
18
19
474 *pretiosum* females (i.e., both directly treated with EO or from F₁ generation) was almost halved in
20
21
475 presence of *A. sativum* or *M. piperita* EOs (Parreira et al., 2019). Yotavong et al. (2015) noted that
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23
476 thymol could influence some biological parameters of the progeny of the parasitoid *C. plutellae*, at
24
25
477 sublethal doses, like the emergence rate and the larval-pupal developmental time. However, there was
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27
478 no impact on detoxification enzymes (cytochrome P450 and carboxylesterase activities) (Yotavong
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29
479 et al., 2015).

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31
480 Lastly, sublethal concentrations of EOs (LC_{30}) can cause consequences in the digestive system
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33
481 of the parasitoid *H. hebetor*, decreasing the enzymatic activity, but not the protein content, in this
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35
482 parasitoid wasp (Asadi et al., 2021).

483 484 **4. Insect pollinators**

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

491 resins. This aspect highlights the need to assess the selectivity of EOs to these insect species because
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492 to date few studies focused on the bioactivity of these botanicals toward pollinators.
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493 Despite the high insecticidal activity of *C. citriodora* EO against *Ascia monuste* (Godart)
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494 (Lepidoptera: Pieridae) (LD₅₀ = 20.61 µg/mg) and its selectivity toward the predatory ant *Solenopsis*
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9
495 *saevissima* (Smith) (Hymenoptera: Formicidae), this EO caused high mortality among *Tetragonisca*
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496 *angustula* (Latreille) (Hymenoptera: Meliponini) adult forager bees, an important generalist
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14
497 pollinator species in tropical regions (Ribeiro et al., 2018). Similarly, *Artemisia annua* L. EO is a
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16
498 promising bioinsecticide against *D. hyalinata*, causing a low mortality against the predator ant *S.*
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499 *saevissima* (42 %), while significant toxicity was demonstrated toward the pollinator bee *T. angustula*
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500 (74%) (Seixas et al., 2018b). Therefore, the use of these EOs when the plants are in the flowering
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501 stage and constantly visited by bees, should be avoided. The absence of physiological selectivity of
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502 EOs, similarly to many synthetic commercial insecticides, does not preclude their use, although it
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503 should be considered under open field conditions. Nevertheless, some botanical extracts
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504 demonstrated good selectivity against stingless bees. In contrast with the previous results, when adult
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505 stingless bees, *Nannotrigona aff. testaceicornis* (Lepelletier) (Hymenoptera: Meliponini), were
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36
506 exposed to synthetic insecticides, *L. sidoides* EO or its major compounds in contact toxicity trials
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38
507 designed to evaluate the lethal and sublethal (i.e., locomotion and flight orientation) effects, the EO
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41
508 and its constituents demonstrated the lowest acute toxicity to forager worker bees, producing no
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509 effects on their locomotion and orientation ability (Matos et al., 2021). Furthermore, the authors
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510 reported that *N. testaceicornis* avoided *L. sidoides* EO and its major constituent thymol in arena trials,
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48
511 suggesting that this non-target species was repelled by the EO presence (Matos et al., 2021).
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512 Consistent with the toxic activity reported for *T. angustula*, EOs can also impact the survival
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53
513 and behavior of the honeybee *Apis mellifera* L. (Hymenoptera: Apidae). Honeybees are beneficial
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514 and economically important insects, having a major impact on crop production because they represent
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58
515 80% of insect pollinators, apart from the market for honey and beeswax. *A. mellifera* is a recognized
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516 bioindicator species since it is very sensitive and greatly affected by environmental changes and
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517 pollutants, as well as by pesticide presence (Burger, 2006). Melo et al. (2018) reported that *L. gracilis*
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518 EOs and their major compounds (i.e., thymol and carvacrol) were effective against the target species
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519 *D. hyalinata*; however, these EOs were not selective to *A. mellifera* L. nor to *Polybia micans* Ducke
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6
520 (Hymenoptera: Vespidae), because in topical toxicity trials these botanicals (i.e., applied at the LD₈₀
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521 for *D. hyalinata*) caused significant mortality (> 80%) for both non-target species (Melo et al., 2018).
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12 In bees, susceptibility towards an EO appears to be influenced by the exposed species rather
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14
523 than the EO. *Apis mellifera* foragers exposed to ginger, mint, oregano, and thyme EOs were less
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524 tolerant than *Trigona hyalinata* (Lepeletier) (Hymenoptera: Apidae) foragers (da Silva et al., 2020).
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525 Conversely, oregano and thyme EOs applied at sublethal doses had negative impact on the distance
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526 traveled, the movement speed and the number of stops by the stingless bee whereas, on *A. mellifera*
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527 foragers only oregano EO showed similar effects (da Silva et al., 2020). The walking activity of *A.*
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528 *mellifera* was negatively affected by eucalyptus EO, as well as neem seed kernel oil, which also
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529 showed a repellent effect towards honeybee foragers (Xavier et al., 2015).
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530 On the other hand, some EOs (eucalyptus, camphor) or single compounds (i.e., thymol and
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531 menthol) are commonly used in commercial acaricide formulations (i.e., ApiLife Var[®] and
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36
532 Apiguard[®]) for *Varroa destructor* (Anderson & Trueman) (Mesostigmata: Varroidae) control, despite
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533 some moderate sub-lethal effects towards honeybees may raise some questions about their presumed
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534 complete harmlessness. Gashout et al., (2015) reported that among different EO compounds tested
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535 against the varroa mite, thymol and menthol had the lowest and the highest LC₅₀ against both adult
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536 bees and larvae, respectively (adults: 210.3 and 523.5 µg/bee; larvae: 150.7 and 382.8 µg /larva).
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537 Furthermore, low concentration of EOs or single compounds (i.e., thymol and carvacrol) may also
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538 impact on the physiology of honeybees, mainly at nervous system level by causing an increase of
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539 acetylcholinesterase and glutathione S-transferase activities (Clavan et al., 2020), as well as EO
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540 compounds can be accumulate in their bodies by both adult bees and larvae (Sammataro et al., 2009).
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541 In the last decade, nanotechnologies strongly influenced research on the formulation of novel
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642 insecticides, both synthetic and natural (de Oliveira et al., 2014; Vurro et al., 2019). Acute toxicity of
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543 peppermint EO and its alginate-based nanoemulsion were recently evaluated against worker bees in
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544 oral and contact toxicity trials by [Youssef and Abdelmegeed \(2021\)](#); nanoemulsion was more toxic
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545 on *A. mellifera* than their crude materials both in contact ($LC_{50} = 5471.13$ and $11,895.65$ ppm,
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6
546 respectively) and oral toxicity trials ($LC_{50} = 2629.85$ and 4246.84 ppm, respectively). Furthermore,
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547 both nanoemulsions and crude EO have biochemical and physiological effects on honeybee workers,
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548 altering amylase, total protein, and lipid contents ([Youssef and Abdelmegeed, 2021](#)).
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5. Soil invertebrates

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551 Among soil invertebrates, most of the studies aimed at the evaluation of the side-effects of EOs used
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552 earthworms as the bioassay species. Among this group, ecotoxicology tests mainly involved the non-
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553 target species *Eisenia fetida* (Savigny) (Haplotaxida: Lumbricidae)s (e.g., [Kang et al., 2022](#); [Nenaah](#)
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554 [et al., 2022](#); [Pavela et al., 2019](#); [Sánchez-Gómez et al., 2022](#); [da Silva Sá et al., 2022](#)). The acute
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555 toxicity of EOs against target crop pests and the non-target earthworm *E. fetida* are presented in **Table**
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556 **3**.
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557 Commonly the EOs have little to no effects against this non-target species; as an example,
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558 *Stevia rebaudiana* (Bertoni) EO was effective against the aphid *Metopolophium dirhodum* (Walker)
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559 (Hemiptera: Aphididae), while it had no effect towards non-target *E. fetida* adults ([Benelli et al.,](#)
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560 [2020b](#)). Similarly, [Pavela et al. \(2020b\)](#) demonstrated that the EOs extracted from *Oliveria*
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561 *decumbens* Vent., *Thymus daenensis* Celak *Satureja sahendica* Bornm., *S. khuzistanica* Jamzad and
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562 *S. rechingeri* Jamzad, effective insecticides against both moth and larvae of *Spodoptera littoralis*
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563 (Boisduval) (Lepidoptera: Noctuidae), were slightly toxic towards *E. fetida* when applied at 200 mg
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564 kg^{-1} of soil, while the positive control α -cypermethrin induced complete mortality at a very low
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565 concentration ($0.1 \text{ mg} \times \text{kg}^{-1}$ of soil) ([Pavela et al., 2020b](#)).
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566 The commercial insecticide α -cypermethrin had a stronger impact on the survival of
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567 earthworms compared to several EOs, which appears to selectively favor *E. fetida* ([Benelli et al.,](#)
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568 [2020a, 2019a, 2019b, 2019d, 2018b](#); [Pavela et al., 2020a](#); [Žabka et al., 2021](#)). Similarly, two
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569 organophosphate insecticides (i.e., monocrotophos and temephos) had a stronger impact on the
1 survival, developmental rate, weight, and enzymatic activity of two earthworms *E. fetida* and
570 *Eudrilus eugeniae* (Kinberg) (Haplotaxida: Eudrilidae) than the EO extracted from *Piper betle* L.;
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4
571 the LC₅₀ observed for the two organophosphates were at least 775-fold lower than that estimated for
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572 the EO (Vasantha-Srinivasan et al., 2018, 2016). Furthermore, monocrotophos and temephos added
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573 in the soil repelled both earthworm species, whereas *P. betle* EO was attractive (Vasantha-Srinivasan
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574 et al., 2018). Similar results were also described by Murfadunnisa et al. (2019) who noted that
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575 *Sphaeranthus amaranthoides* Burm. f. (Asteraceae) EO caused no toxicity against *E. eugeniae* at the
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576 maximum dose of 1000 and 1500 ppm, while the synthetic chemical monocrotophos heavily affected
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577 the earthworm survival.
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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 (LC₅₀ = 10.3 µg cm⁻²) compared to crude EO (LC₅₀ = 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) (EC₅₀ = 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

595 in the next years; therefore, the ecotoxicological evaluation of this kind of pesticides is fundamental
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596 to understand their environmental impact. Nowadays, few studies, compared to the huge amount of
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597 research on EO bioactivity against crop pests, focused on the side effects toward natural enemies.
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6 Knowledge about non-target effects is needed to boost the large-scale industrial production of EO-
598 based pesticides but also due to regulatory strictness. However, it is quite surprising that a very limited
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599 number of papers tested the side effects of commercial biopesticides containing EO as active
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600 ingredients, that have been on the market for at least a decade. Indeed, these commercial products
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601 might be used by farmers for integrated pest management programs involving biopesticides and BCA;
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602 nevertheless, the compatibility and economic sustainability of these two techniques should be
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603 addressed before suggesting their coupled application.
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605 Generally, despite the usual lower efficacy of botanicals compared to conventional pesticides, the use
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606 of botanicals may be a valid alternative in terms of crop yields. Indeed, crops can tolerate a certain
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607 amount of pest damage and the selectivity of plant-based pesticide can ensure pest reduction through
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608 conservation of natural enemies and non-target species (Tembo et al., 2018). The selectivity of
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609 botanicals, including EOs-based pesticides, can be obtained following different paths such as: (i)
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610 timing of pest treatment; (ii) timing of natural enemies' release; (iii) correct choice of pesticide
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611 formulation according to the target pest and beneficials; iv) use of different types of formulations
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612 (e.g., nano vs. traditional). A holistic view of pest control that considers plant protection,
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613 environment, human health, and economic aspects will be able to facilitate the integration of
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614 biopesticides into agro-ecologically sustainable crop production systems.
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615 From a commercial and marketing standpoint, only those effective EOs coming from plants
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616 which are cultivated on a large scale and that are obtainable in middle-high yield (> 1% on a dry
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617 weight basis; the price of an EO is inversely linked to the yield), thus offering a cost-effective raw
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618 material (often derived from cultivation waste), should be used for agrochemical industries. To
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619 improve the latter parameter, new effective extraction techniques (e.g., MAE, enzyme-assisted
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620 distillation, etc.) capable of boosting the release of EO constituents from the plant secretory structures
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621 should be more explored in the future. From a registration perspective, the EOs which are generally
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622 recognized as safe (GRAS) from the principal authorities (i.e., FDA, EFSA, EPA, etc.) or are derived
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623 from plants with documented use as a food (so that they do not pose particular risk from their usage)
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624 should be preferred to the ones coming from plants subjected to some restrictions (e.g., toxic plants).
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625 Finally, more research is needed on the development and evaluation of the ecotoxicological effects
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626 of nanocarriers (e.g., micro- and nano-emulsions, nanoparticles made with plant polymers, liposomes,
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627 protein baits) able to incorporate these EOs and spread them on crops in an eco-sustainable way
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628 (Pavoni et al., 2019; Sánchez-Gomez et al., 2022).

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629 To date very few studies evaluated the impact of EO and their formulations toward pollinators;
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630 this aspect is crucial to understand the ecological impact of biopesticides in the fields, but it seems
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631 quite neglected by scientists. Pollination and pollinator losses are key topics in modern agriculture,
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632 as well as from an ecological point of view. Future studies should focus on the possible side effects
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633 of EOs toward these species to evaluate their eco-safety potential.
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634 The modest number of studies exploring non-targeted effects of EO-based pesticides also
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635 share shortcomings common in studies with conventional insecticides, despite recent shift in that
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636 regard relative to the latter. Two important shortcomings in such studies merit particular attention: (i)
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637 the common assumption of a monophasic response with an increase in EO dose or concentration, and
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638 (ii) the study focus on isolated species. The first shortcoming neglects the possibility of biphasic
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639 concentration-response taking place, consistent with the hormesis phenomenon, in which exposure
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640 levels below the no-observed-adverse-effect-level (sub-NOEL) lead to a stimulatory response
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641 potential benefit to the exposed organism (e.g., non-targeted species) ([Agathokleus and Calabrese,](#)
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642 [2020](#); [Belz and Duke, 2022](#)). The potential importance of this phenomenon for pest management and
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643 environmental impact has been increasingly recognized for a broad range of anthropogenic stressors,
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644 including insecticides ([Guedes and Cutler, 2014](#), [Guedes et al., 2016, 2022](#)), but largely neglected for
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645 plant-based compounds, such as EO-based pesticides ([Haddi et al., 2020](#)).

646 The second shortcoming on the current studies is the focus on isolated species, which although
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647 understandable based on a cost-effective experimental standpoint, neglect the fact that isolated
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648 species do not exist in natural environments and species interactions are prevalent. Thus, more
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649 realistic studies exploring the invertebrate communities are necessary not only to ascertain the field
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650 efficacy of EO-based formulations, but particularly to assess their potential non-target impact
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651 cascading from directly exposed targeted species to potentially directly and indirectly affected non-
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652 target species (Cutler et al. 2022; Guedes et al. 2016, 2022b). Conceptual frameworks such as the
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653 stress-response pathway are useful in that regard, although still underexplored even for the assessment
13
654 of environmental impacts of conventional pesticides (Guedes et al., 2017). Thus, the rethinking and
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655 expanding of the scope of studies with EO-based insecticidal and acaricidal formulations is a need
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656 worth pursuing.
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657 Therefore, national and international regulators are now paying more attention about the
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658 ecotoxicological impact of pesticides, including biopesticides based on plant-borne a.i., to ensure
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659 their environmental safety. In the last decades, the authorization process of botanicals has been greatly
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660 facilitated in the USA, by instituting exemptions from the normal regulatory approval process
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661 required for synthetic pesticides to certain EOs and their major constituents (Isman 2020). A similar
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662 approach has been used also by EU legislators, although with many more limitations and far less
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663 success (Vekemans & Marchand, 2020). In this regard, it should be kept in mind that the European
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664 legislation concerning plant protection products (PPP) (regulation (EC) N° 1107/2009) is quite
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665 unclear about the definition of PPP admitted in organic agriculture, botanical-based products
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666 included, thus the registration of green/biopesticides often faces insurmountable obstacles throughout
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667 the whole authorization process as a consequence (Vekemans & Marchand, 2020). In this scenario,
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668 research on non-target impact of botanical-based pesticides may improve the knowledge and the
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669 awareness about their ecotoxicological safety both among companies and industries, as well as within
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670 the regulator agencies, promoting and supporting further registration and commercialization.
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CRediT author statement

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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	<i>Allium sativum</i>	<i>Nesidiocoris tenuis</i>	<i>Tuta absoluta</i>	R	A	Survival; Fertility	-	+	Ricupero et al. 2022
Apiaceae	<i>Coriandrum sativum</i>	<i>Cyrtorhinus lividipennis</i>	<i>Nilaparvata lugens</i>	R	N, A	Attractivity; Orientation		+	Liu et al., 2019
	<i>Ferula gummosa</i>	<i>Orius albidipennis</i>	<i>Bemisia tabaci</i>	F	A	LC ₅₀ = 3.467 µL L ⁻¹	-		Zandi-Sohani et al., 2018
	<i>Heracleum persicum</i>	<i>Hippodamia variegata</i>	<i>Rhopalosiphum padi</i> , <i>Sitobion avenae</i> , <i>Schizaphis graminum</i> , <i>Metopolophium dirhodum</i>	I	E, N	Mortality; Life history traits	-	+	Shaltoki et al., 2022
Apocynaceae	<i>Nerium indicum</i>	<i>Cyrtorhinus lividipennis</i>	<i>Nilaparvata lugens</i>	R	N, A	Attractivity; Orientation		+	Liu et al., 2019
Asteraceae	<i>Achillea millefolium</i>	<i>Hippodamia variegata</i>	<i>Rhopalosiphum padi</i> , <i>Sitobion avenae</i> , <i>Schizaphis graminum</i> , <i>Metopolophium dirhodum</i>	I	E, N	Mortality; Life history traits	-	+	Shaltoki et al., 2022
	<i>Artemisia sieberi</i>	<i>Orius albidipennis</i>	<i>Bemisia tabaci</i>	F	A	LC ₅₀ = 0.621 µL L ⁻¹	+		Zandi-Sohani et al., 2018
	<i>Vanillosmopsis arborea</i>	<i>Euborellia annulipes</i>	<i>Spodoptera frugiperda</i>	T	N	LD ₅₀ = 160.2 mg mL ⁻¹	+		Alves et al., 2022
Boraginaceae	<i>Varronia curassavica</i>	<i>Ceraeochrysa cubana</i>	<i>Myzus persicae</i> , <i>T urticae</i>	R	N	Mortality	-		Andrade et al., 2021
Brassicaceae	<i>Brassica nigra</i>	<i>Podisus maculiventris</i>	<i>Spodoptera exigua</i>	T, I	N, A	Mortality	-		Tavares et al., 2019
Euphorbiaceae	<i>Croton grewoides</i>	<i>Neoseiulus californicus</i>	<i>Tetranychus urticae</i>	R, I	A	LC ₅₀ = 3.26 µL mL ⁻¹	-		de Santana et al., 2021
	<i>Croton rhamnifolioides</i>	<i>Neoseiulus californicus</i>	<i>Tetranychus urticae</i>	R, I	A	LC ₅₀ = 1.14 µL mL ⁻¹	-		de Santana et al., 2021
Geraniaceae	<i>Pelargonium graveolens</i>	<i>Orius albidipennis</i>	<i>Bemisia tabaci</i>	F	A	LC ₅₀ = 0.954 µL L ⁻¹	+		Zandi-Sohani et al., 2018
Lamiaceae	<i>Melissa officinalis</i>	<i>Neoseiulus barkeri</i>	<i>Tetranychus urticae</i>	T	E, A	Mortality; Food consumption; Fecundity	+	+	Abdel Kader et al., 2015
		<i>Typhlodromips swirskii</i>	<i>Tetranychus urticae</i>	T	E, A	Mortality; Food consumption; Fecundity	+	+	Abdel Kader et al., 2015
		<i>Neoseiulus californicus</i>	<i>Tetranychus urticae</i>	T, F	E, N, A	Mortality	-		Momen et al., 2014
	<i>Mentha longifolia</i>	<i>Coccinella undecimpunctata</i>	<i>Aphis punicae</i>	R	N	LC ₅₀ = 8.737 µg mL ⁻¹	+		Sayed et al., 2022
	<i>Mentha piperita</i>	<i>Coccinella undecimpunctata</i>	<i>Aphis punicae</i>	R	N	LC ₅₀ = 10.334 µg mL ⁻¹	+		Sayed et al., 2022
	<i>Mentha pulegium</i>	<i>Adalia bipunctata</i>	<i>Acyrtosiphon pisum</i> , <i>Aphis fabae</i> , <i>Macrosiphoniella</i>	F	A	LC ₅₀ = 0.19 µL L ⁻¹	+		Kimbaris et al., 2010

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

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

	<i>Lippia microphylla</i>	<i>Euborellia annulipes</i>	<i>Spodoptera frugiperda</i>	<i>T</i>	<i>N</i>	LD ₅₀ = 134.67 mg mL ⁻¹	+		Alves et al., 2022
	<i>Lippia sidoides</i>	<i>Neoseiulus californicus</i>	<i>Tetranychus urticae</i>	<i>R, I</i>	<i>A</i>	LC ₅₀ = 0.78 µL mL ⁻¹	-		de Santana et al., 2021
	<i>Lippia sidoides</i>	<i>Podisus nigrispinus</i>	<i>Spodoptera frugiperda</i>	<i>T</i>	<i>N</i>	LD ₅₀ = 28.43 mg g ⁻¹ ; LT ₅₀ = 119 h; Locomotory activity; Repellence	+	+	Lima et al., 2020
Zingiberaceae	<i>Alpinia officinarum</i>	<i>Cyrtorhinus lividipennis</i>	<i>Nilaparvata lugens</i>	<i>R</i>	<i>N, A</i>	Attractivity; Orientation		+	Liu et al., 2019
	<i>Curcuma longa</i>	<i>Podisus maculiventris</i>	<i>Spodoptera exigua</i>	<i>T, I</i>	<i>N, A</i>	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	<i>Allium sativum</i>	<i>Trichogramma galloi</i>		<i>C</i>	<i>E</i>	Life history traits; transgenerational effect	+		Parreira et al., 2018
		<i>Trichogramma pretiosum</i>		<i>R</i>	<i>E, A</i>	Life history traits; transgenerational effect	+		Parreira et al., 2019
Anacardiaceae	<i>Schinus molle</i> var. <i>areira</i>	<i>Trissolcus basal</i>	<i>Nezara viridula</i>	<i>F, T</i>	<i>A</i>	LC ₅₀ = 75.69 µg mL ⁻¹ /0.56 µg cm ⁻² ; Oviposition	+	-	Werdirn González et al., 2013
Apiaceae	<i>Carum carvi</i>	<i>Habrobracon hebetor</i>		<i>F</i>	<i>A</i>	LC ₅₀ = 0.340 µL L ⁻¹ ; Life history traits	+	+	Razmjou et al., 2018
	<i>Coriandrum sativum</i>	<i>Cotesia glomerata</i>	<i>Plutella xylostella</i>	<i>F</i>	<i>A</i>	LD ₅₀ = 5.52 mg/filter paper	-		Yi et al., 2007
	<i>Ferula assafoetida</i>	<i>Trichogramma embryophagum</i> <i>Trichogramma evanescens</i>	<i>Ephestia kuehniella</i>	<i>F</i>	<i>A</i>	LC ₅₀ = 1758 ppm; Life history traits; Mating behavior	-	+	Poorjavad et al., 2014
	<i>Foeniculum vulgare</i>	<i>Habrobracon hebetor</i>		<i>F</i>	<i>A</i>	LC ₅₀ = 0.48 mL L ⁻¹ ; Life history traits	+	+	Ahmadpour et al., 2021
	<i>Heracleum persicum</i>	<i>Habrobracon hebetor</i>		<i>F</i>	<i>A</i>	LC ₅₀ = 3.416 µL L ⁻¹ ; Life history traits	-	+	Razmjou et al., 2018
Asteraceae	<i>Achillea millefolium</i>	<i>Habrobracon hebetor</i>		<i>F</i>	<i>A</i>	LC ₅₀ = 1.68 mL L ⁻¹ ; Life history traits	-	+	Ahmadpour et al., 2021
	<i>Artemisia vulgaris</i>	<i>Cotesia glomerata</i>	<i>Plutella xylostella</i>	<i>F</i>	<i>A</i>	LD ₅₀ = 2.18 mg/filter paper	+		Yi et al., 2007
	<i>Artemisia campestris</i>	<i>Dinarmus basal</i>	<i>Callosobruchus maculatus</i> - <i>Bruchus rufimanus</i>	<i>F</i>	<i>A</i>	Adult emergence		+	Titouhi et al., 2017
			<i>Callosobruchus maculatus</i> - <i>Bruchus rufimanus</i>	<i>F</i>	<i>A</i>	Adult emergence		+	Titouhi et al., 2017
	<i>Artemisia herba-alba</i>	<i>Dinarmus basal</i>	<i>Callosobruchus maculatus</i> - <i>Bruchus rufimanus</i>	<i>F</i>	<i>A</i>	Adult emergence		+	Titouhi et al., 2017
		<i>Triaspis luteipes</i>	<i>Callosobruchus maculatus</i> - <i>Bruchus rufimanus</i>	<i>F</i>	<i>A</i>	Adult emergence		+	Titouhi et al., 2017
Atherospermataceae	<i>Laurelia sempervirens</i>	<i>Encarsia formosa</i>	<i>Trialeurodes vaporariorum</i>	<i>F</i>	<i>A</i>	LC ₅₀ = 0.86 µL L ⁻¹ air; LT; Fecundity	+	+	Zapata et al., 2016
Cupressaceae	<i>Thuja occidentalis</i>	<i>Cotesia glomerata</i>	<i>Plutella xylostella</i>	<i>F</i>	<i>A</i>	LD ₅₀ = 2.28 mg/filter paper	+		Yi et al., 2007
Lamiaceae	<i>Hyptis marrubiioides</i>	<i>Trichogramma pretiosum</i> ,	<i>Spodoptera frugiperda</i>	<i>R</i>	<i>A</i>	Survival; Fecundity	-	-	Bibiano et al., 2022
	<i>Hyptis spicigera</i>	<i>Dinarmus basal</i>		<i>F</i>	<i>L, A</i>	Mortality; Oviposition	+	+	Sanon et al., 2010
	<i>Hyptis suaevolens</i>	<i>Dinarmus basal</i>		<i>F</i>	<i>L, A</i>	Mortality; Oviposition	+	+	Sanon et al., 2010
	<i>Lavandula angustifolia</i>	<i>Cotesia glomerata</i>	<i>Plutella xylostella</i>	<i>F</i>	<i>A</i>	LD ₅₀ = 8.51 mg/filter paper	-		Yi et al., 2007

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

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

	<i>Lippia organoides</i>	<i>Trichogramma pretiosum</i> ,	<i>T</i>	<i>A</i>	LC ₅₀ = 0.43%; Oviposition	-	+	Sombra et al., 2022
Zingiberaceae	<i>Zingiber officinale</i>	<i>Trichogramma galloi</i>	<i>R</i>	<i>E</i>	Life history traits; transgenerational effect		+	Parreira et al., 2018
		<i>Trichogramma pretiosum</i>	<i>R</i>	<i>E, A</i>	Life history traits; transgenerational effect		+	Parreira et al., 2019
		<i>Trichospilus pupivorus</i>	<i>R</i>	<i>A</i>	LC ₅₀ = 8.16%		+	Moreira da Silva et al., 2020
		<i>Diaphania hyalinata</i>						

* *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 \pm 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	<i>E. fetida</i> mortality (%)	References
<i>Deverra tortuosa</i>	Apiaceae	<i>Callosobruchus maculatus</i> (LC ₅₀ = 23.1 $\mu\text{g cm}^{-2}$)	200 mg kg ⁻¹ soil	0.0 \pm 0.0 (*10 days)	Almadiy et al., 2022
<i>Ocimum sanctum</i>	Lamiaceae	<i>Spodoptera littoralis</i> (LD ₅₀ = 39.3 $\mu\text{g larva}^{-1}$)	500 mg kg ⁻¹ soil	10.0 \pm 5.0	Žabka et al., 2021
<i>Ledum palustre</i>	Ericaceae	<i>Spodoptera littoralis</i> (LD ₅₀ = 117.2 $\mu\text{g larva}^{-1}$)	250 mg kg ⁻¹ soil	5.0 \pm 5.0	Benelli et al., 2020a
<i>Stevia rebaudiana</i>	Asteraceae	<i>Metopolophium dirhodum</i> (LC ₅₀ = 5.1 mL L ⁻¹)	200 mg kg ⁻¹ soil	0.0 \pm 0.0	Benelli et al., 2020b
<i>Ferula assa-foetida</i>	Apiaceae	<i>Spodoptera littoralis</i> (LD ₅₀ = 29.3 $\mu\text{g larva}^{-1}$)	200 mg kg ⁻¹ soil	0.0 \pm 0.0	Pavela et al., 2020a
<i>Ferula gummosa</i>	Apiaceae	<i>Spodoptera littoralis</i> (LD ₅₀ = 124.4 $\mu\text{g larva}^{-1}$)	200 mg kg ⁻¹ soil	0.0 \pm 0.0	
<i>Oliveria decumbens</i>	Apiaceae	<i>Spodoptera littoralis</i> (LD ₅₀ = 7.4 $\mu\text{g larva}^{-1}$)	200 mg kg ⁻¹ soil	0.0 \pm 0.0	Pavela et al., 2020b
<i>Thymus daenensis</i>	Lamiaceae	<i>Spodoptera littoralis</i> (LD ₅₀ = 9.6 $\mu\text{g larva}^{-1}$)	200 mg kg ⁻¹ soil	7.5 \pm 4.3	
<i>Satureja sahendica</i>	Lamiaceae	<i>Spodoptera littoralis</i> (LD ₅₀ = 23.1 $\mu\text{g larva}^{-1}$)	200 mg kg ⁻¹ soil	0.0 \pm 0.0	
<i>Satureja khuzistanica</i>	Lamiaceae	<i>Spodoptera littoralis</i> (LD ₅₀ = 8.9 $\mu\text{g larva}^{-1}$)	200 mg kg ⁻¹ soil	0.0 \pm 0.0	
<i>Satureja rechingeri</i>	Lamiaceae	<i>Spodoptera littoralis</i> (LD ₅₀ = 9.4 $\mu\text{g larva}^{-1}$)	200 mg kg ⁻¹ soil	0.0 \pm 0.0	

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

<i>Foeniculum vulgare</i>	Apiaceae	<i>Myzus persicae</i> (LC ₅₀ = 0.6 mL L ⁻¹)	240.7 mg kg ⁻¹ soil	12.5 ± 5.0	Pavela, 2018
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