Chemical Composition and Broad-Spectrum Insecticidal Activity of the Flower Essential Oil from an Ancient Sicilian Food Plant, *Ridolfia segetum*

Natale Badalamenti 1,*, Vincenzo Ilardi 2, Maurizio Bruno 1,*, Roman Pavela 3,4,*, Maria C. Boukouvala 5, Nickolas G. Kavallieratos 5, Filippo Maggi 6,*, Angelo Canale 7 and Giovanni Benelli 7,*

1 Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Viale delle Scienze, Parco d’Orleans II, 90128 Palermo, Italy; natale.badalamenti@unipa.it (N.B.); maurizio.bruno@unipa.it (M.B.)
2 Department of Earth and Marine Sciences (DISTeM), University of Palermo, Via Archirafi 26, 90128 Palermo, Italy; vincenzo.ilardi@unipa.it
3 Crop Research Institute, Dmowska 507, 161 06 Prague 6, Ruzyn, Czech Republic; pavela@vurv.cz
4 Department of Plant Protection, Czech University of Life Sciences Prague, Kamýcka 129, 165 00 Praha 6, Suchdol, Czech Republic
5 Laboratory of Agricultural Zoology and Entomology, Department of Crop Science, Agricultural University of Athens, 75 Iera Odos Str, 11855 Athens, Attica, Greece; mbouk@aua.gr (M.C.B.); nick_kaval@aua.gr (N.G.K.)
6 School of Pharmacy, University of Camerino, Via Sant’Agostino 1, 62032 Camerino, Italy
7 Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy; angelo.canale@unipi.it (A.C.); giovanni.benelli@unipi.it (G.B.)
* Correspondence: filippo.maggi@unicam.it; Tel.: +39-0737-404506

Abstract: Several species of the family Apiaceae are aromatic herbs that produce essential oils usable on an industrial scale for pharmaceutical, cosmetic, and food purposes. In particular, some essential oils, such as green insecticides for example, may replace synthetic insecticides, keeping most of their efficacy and avoiding environmental pollution or human poisoning. In the present study, we explored the insecticidal potential of *Ridolfia segetum* (L.) Moris essential oil (EO) against three different pests: *Culex quinquefasciatus* Say, *Musca domestica* L., and *Spodoptera littoralis* (Boisdurval). For this purpose, the EO was obtained by hydrodistillation of flowers and its composition was achieved by gas chromatography/flame ionization detection (GC/FID) and gas chromatography/mass spectrometry (GC/MS). This EO was rich in α-phellandrene (49.3%), β-phellandrene (9.2%), terpinolene (20.7%), and piperitenone oxide (5.9%). Concerning the mosquitocidal efficacy, the EO showed noteworthy toxicity against *C. quinquefasciatus* 3rd instar larvae, with a LC̅50 = 27.1 µL L−1 and LC̅90 = 42.5 µL L−1. Regarding *M. domestica*, a different toxicity of the *R. segetum* EO was found on male and female flies, calculating LD̅30 values of 10.5 and 30.8 µg adult−1, respectively. The EO was also toxic to *S. littoralis* 3rd instar larvae, achieving LD̅30 and LD̅90 values of 37.9 and 99.6 µg larva−1, respectively. Overall, this flower EO, extracted from a traditional Sicilian food plant, merits further investigation for the development of green insecticide formulations to be used in real world conditions, pending a careful assessment of non-target toxicity on beneficial organisms.

Keywords: *Culex quinquefasciatus*; green pesticides; common housefly; mosquito control; moth pest; *Musca domestica*; *Spodoptera littoralis*

1. Introduction

Arthropods include numerous agricultural pests, as well as key species that play a major role as vectors of pathogens, including malaria, dengue, Zika virus, lymphatic filariasis, Lyme disease, and many others [1,2]. Their control has a long history, and it is still routinely achieved through massive applications of synthetic insecticides [3–5], with severe effects on human health and the environment, long-term sub-lethal effects [6–10].
and the quick development of resistance in the targeted species [11–13]. In the attempt to discover novel active ingredients for insecticidal, acaricidal, and repellent formulations, botanical products have been largely investigated [14–16], outlining their scarce toxicity to vertebrates [17], as well as their multiple modes of action on arthropods, which strongly reduce the risk of resistance development [18,19]. Although the commercialization of botanical insecticides (BIs) seems complex and slow for the time being [17,20,21], research needs to be intensified, especially in terms of phytochemical standardization of the active ingredients, formulation of BIs to ensure long-term effectiveness, and the search for new plant species as potential sources of active substances for BIs [16,22]. In particular, considering the decreasing number of authorized active ingredients of synthetic insecticides and the greening of agricultural production, BIs are of growing relevance among the preferred products for plant protection purposes [17,20,21]. In this scenario, research focusing on the largely unexplored insecticidal potential of aromatic food plant species growing in arid environments is of special interest [23], with potential connections to the local agricultural economy of Mediterranean countries.

*Ridolfia segetum* (L.) Moris (Apiales: Apiaceae) is an annual plant (Teraofita), 3–8 dm high, hairless, and with an unpleasant odor. It is a Mediterranean species found commonly in cereal fields and temporarily uncultivated grounds in southern Europe, Turkey, the Middle East, and North Africa [24]. In Italy, it is present in all central and southern regions, from Liguria, Tuscany, and Marche, to Sardinia, and Sicily [25,26].

In Morocco, it is cooked as a vegetable in several recipes including tajines, couscous, and beqoula [27], whereas in Sicily, this plant, locally known as “finoctiui anitu,” is consumed raw in salads and cooked [28]; its roots and stems are eaten to treat gastric acidity [29].

Earlier research focused on the composition of the essential oils of other accessions, growing in Europe and North Africa. Some biological properties of *R. segetum* essential oil (EO) have also been reported. The EO of the flowers collected in Portugal showed anti-inflammatory and antioxidant activities [30] and, recently, strong antitumor potential with high capability to inhibit proliferation and induce apoptosis [31]. Jabrane et al. [32,33] and Ben Jannet and Mighri [34] showed the antibacterial activities of the EOs from plants collected in Tunisia, whereas the EO from the Sardinian population was shown to inhibit HIV-1 RT RDDP activity in a dose-dependent manner [35].

In the frame of our ongoing research on Mediterranean species belonging to the Apiaceae family [36–41], we analyzed the chemical composition of the flower EO of a Sicilian accession of *R. segetum*, not investigated so far, and evaluated its insecticidal activity on a panel of insect species of high economic importance, i.e., the lymphatic filariasis vector *Culex quinquefasciatus* Say (Diptera: Culicidae)—for which the development of novel and sustainable control tools is urgently needed [37,42]—the common housefly, *Musca domestica* L. (Diptera: Muscidae), which is a noxious insect being able to transmit a large number of microbial pathogens [43], and the African cotton leafworm, *Spodoptera littoralis* (Boisdruval) (Lepidoptera: Noctuidae), which is currently considered one of the most polyphagous moth species attacking highly diverse crops tropic and sub-tropic regions, and is listed as an A2 quarantine pest [44]. For mosquitoes and moths, the third larval instar was targeted, while for houseflies we examined the EO toxicity on adults, assessing the potential difference in EO effectiveness on male and female flies.

2. Materials and Methods
2.1. Plant Material

Flowers of *R. segetum* were collected in June 2019, at Borgo Eras, Collesano, Palermo (Sicily) at 600 m of altitude (37°53′34.84″ N and 13°54′41.08″ E). Typical specimens (PAL 109714), collected and identified by Prof. Vincenzo Ilardi, have been deposited in Herbarium Mediterranean Panormitanum of the “Orto Botanico,” Palermo, Italy.
2.2. Essential Oil Extraction

350 g of flowers of *R. segetum* were subjected to hydrodistillation for 3 h using a Clevenger type apparatus [45]. The EO (yield 0.9\% (v/w)) was dried with anhydrous sodium sulphate, filtered, and stored in the freezer at −20 °C, until the time of analysis.

2.3. GC-FID Analysis of the Essential Oil

An Agilent 4890D gas chromatograph coupled with an ionization flame detector (FID) was used. The separation stationary phase was represented by a HP-5 capillary column (5% phenylmethylpolysiloxane, 25 m, 0.32 mm i.d.; 0.17 \( \mu \)m f.t.) (Agilent, Folsom, CA, USA). The mobile phase was helium (99.999\%) flowing at 1.0 mL/min. The oven temperature programmer was as follows: 60 °C isothermal for 5 min, then ramp (4 °C/min) to 220 °C, and ramp (11 °C/min) to 280 °C. The EO was diluted to 1:100 in hexane and the volume injected was 1 \( \mu \)L in split mode (1:34). The injector and detector temperatures were set to 280 °C. A commercial mix of \( n \)-alkanes (C\(_8\)–C\(_{30}\)) purchased from Supelco (Bellefonte, CA, USA) was used to determine the peak linear retention index (RI). Quantitative values, expressed as percentages, were obtained following the procedure by Cecchini et al. [46]; they were the mean of three determinations.

2.4. GC-MS Analysis of the Essential Oil

An Agilent 6890N gas chromatograph equipped with a 5973N single quadrupole mass spectrometer (MS) was employed. The stationary phase was a HP-5MS capillary column (30 m, 0.25 mm i.d., 0.1 \( \mu \)m f.t.) (Agilent). The operative conditions and the mobile phase were the same of those reported above. The injector and transfer line temperatures were 280 and 250°C, respectively. Same dilution of that reported above was injected into the GC-MS system in split mode (1:50). Mass spectra were acquired in electron impact (EI) mode in the range of 29–400 m/z. The identification was carried out by combination of mass spectra (MS) matching and RI overlapping against the Adams [47], NIST 17 [48] and FFNSC 2 [49] libraries. The comparison with available authentic standards (Sigma-Aldrich, Milan, Italy) was also used for \( \alpha \)-pinene, camphene, sabinene, \( \beta \)-pinene, myrcene, \( \alpha \)-phellandrene, \( p \)-cymene, limonene, \( \gamma \)-terpinene, terpinolene, camphor, and terpinen-4-ol.

2.5. Insect Rearing

Populations of *C. quinquefasciatus*, *M. domestica*, and *S. littoralis* were reared following the methods described by Pavela [50–52]. Experimental colonies of mosquitoes from the Crop Research Institute (Prague, Czech Republic) were established. Adult mosquitoes were maintained in entomological cages (40 × 40 × 40 cm) and fed a 10% sucrose solution. Egg hatching was achieved in tap water. Larvae were reared in plastic trays (30 × 35 × 25 cm, about 100 larvae/L). The larvae were fed on dog biscuits and yeast powder at 3:1 (w:w) ratio [50]. All stages were held at 25 ± 2 °C, 65–75% R.H., and a 12:12 h (L:D) photoperiod.

2.6. Insecticidal Activity on *Culex quinquefasciatus*

The *R. segetum* flower EO was diluted in dimethyl sulfoxide (DMSO) and tested against early 3rd instar larvae of *C. quinquefasciatus* following the WHO method [53], with minor changes by Benelli et al. [54]. The larvae were selected and transferred in 25 mL of distilled water. The EO was diluted in DMSO obtaining the following test concentrations: 10, 20, 30, 40, and 50 \( \mu \)L L\(^{-1}\). For experimental treatments, 1 mL of each serial EO dilution was added to 224 mL of distilled water in a 500-mL glass bowl, and shaken lightly to ensure a homogenous test solution. Then, the selected larvae were transferred from distilled water into a bowl of the test solution with a final surface area of 125 cm\(^2\) (25 larvae/beaker). Each concentration was replicated 4 times on groups of 25 larvae each. Distilled water with the same amount of DMSO used for dissolving the EO was the negative control. Larval mortality was recorded after 24 h in a growth chamber (photoperiod 16:8, 24 ± 1 °C). No food was offered to the larvae during the testing and resting phases.
2.7. Insecticidal Activity on Musca domestica

The *R. segetum* flower EO was tested on adult females and males (3–6 days old) through topical application, following Benelli et al. [55]. One µL of acetone (Sigma-Aldrich, Germany) plus the EO at 5, 10, 20, 40, 60, 80, 100, 120, 140, and 160 µg adult⁻¹ (each concentration was tested on 4 groups of 20 males or females each), was applied using a microelectric applicator (Hardy Step Electronic, Brand, Czech Republic) to the pronotum of flies anesthetized with carbon dioxide. The number and range of doses were determined from preliminary experiments. Acetone was the negative control. The flies were moved to a recovery box (10 × 10 × 12 cm, placed in a growth chamber, photoperiod 16:8, 24 ± 1 °C) for 24 h, then mortality was recorded for males and females.

2.8. Insecticidal Activity on Spodoptera littoralis

The *R. segetum* flower EO diluted in acetone was tested through topical application to *S. littoralis* early 3rd instar larvae (weight 20–25 mg larva⁻¹). According to Pavela et al. [23], each larva was treated on the dorsum with 1 µL of acetone containing 20, 30, 40, 50, 70, 90, and 100 µg larva⁻¹ of *R. segetum* EO. Each concentration was replicated 4 times (*n* = 20 larvae replicate⁻¹). The number and range of individual doses were determined from preliminary experiments. Acetone was the negative control. All treated larvae from each replication were transferred to the relevant diet in plastic boxes (10 × 10 × 7 cm) for 24 h and stored in a growth chamber (photoperiod 16:8, 24 ± 1 °C). Each box had a perforated cap to avoid any fumigation effect of the EO or acetone.

2.9. Statistical Analysis

Lethal doses or lethal concentrations were estimated using Probit analysis [56]. The calculation also included a possible correction of mortality according to Abbott [57]. Calculations were performed using statistical software BioStat Pro (version 5.8.9.).

3. Results and Discussion

3.1. Flower Essential Oil Yield and Its Chemical Composition

Hydro-distillation of the flowers of *R. segetum* gave a pale yellow EO with a yield of 0.9% (v/w). Overall, 27 compounds were identified, representing 99.9% of the total compositions. The components are listed in Table 1 according to their retention indices on a HP-5MS column and are classified based on their chemical structures into four classes. This EO was extremely rich in monoterpene hydrocarbons (90.1%). *α*-Phellandrene (49.3%) was, by far, the main component of this class as well as of the EO, followed by terpinolene (20.7%) and *β*-phellandrene (9.2%). These 3 components made up about 80% of the total composition. Oxygenated monoterpenes were present in lower amounts (6.1%), with piperitenone oxide (5.9%) as the main compound of the class, whereas sesquiterpene derivatives were practically absent. Among other compounds, it is worthy to point out the occurrence of a good quantity of dill apiol (3.7%).

Based on composition of EOs of different accessions of *R. segetum* studied so far, it is possible to identify three distinct chemotypes: the first one is largely dominated by monoterpene hydrocarbons, usually *α*-phellandrene, terpinolene, and *p*-cymene [35,58–60]; a second one which contains phenylpropanoids, usually myristicin and/or dillapiole as major compounds [33,34,61]; and a third one rich in dillapiole and *o*-cymene [62].

The composition of *R. segetum* EO clearly indicates that the Sicilian accession belongs to the first chemotype. In fact, the comparison with the EOs from flowers of other populations shows a high similitude with those from Sardinia [(*α*-phellandrene (24.7%), terpinolene (19.9%))] [58], Andalusia, Spain [(*α*-phellandrene (54.7–44.5%), terpinolene (27.6–20.1%))] [59], Castilia la Mancha, Spain [(*α*-phellandrene (33.8–32.0%), terpinolene (21.4–18.0%))] [60] and Kroussia, Tunisia [(*α*-phellandrene (34.7%), terpinolene (23.7%)) [32]. On the other hand, the oil from M’saken, Tunisia [34] and Morocco [61,63], rich in dillapiole and myristicin, showed a completely different profile with respect to our sample.
Table S1 reviews the composition of EOs of different accessions of *R. segetum* studied so far (Supplementary Material).

### Table 1. Composition (%) of the essential oil of flowers of *Ridolfia segetum* collected in Sicily.

<table>
<thead>
<tr>
<th>Components</th>
<th>LRI</th>
<th>LRI Lit.</th>
<th>%</th>
<th>Id.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1   α-Thujene</td>
<td>921</td>
<td>924</td>
<td>0.4</td>
<td>RI, MS</td>
</tr>
<tr>
<td>2   α-Pinene</td>
<td>926</td>
<td>932</td>
<td>3.0</td>
<td>Co-I,RLMS</td>
</tr>
<tr>
<td>3   Camphene</td>
<td>939</td>
<td>946</td>
<td>T</td>
<td>Co-I,RLMS</td>
</tr>
<tr>
<td>4   Sabine 1</td>
<td>965</td>
<td>969</td>
<td>0.5</td>
<td>Co-I,RLMS</td>
</tr>
<tr>
<td>5   β-Pinene</td>
<td>968</td>
<td>974</td>
<td>2.5</td>
<td>Co-I,RLMS</td>
</tr>
<tr>
<td>6   Myrcene</td>
<td>989</td>
<td>988</td>
<td>1.1</td>
<td>Co-I,RLMS</td>
</tr>
<tr>
<td>7   α-Phellandrene</td>
<td>1004</td>
<td>1002</td>
<td>49.3</td>
<td>Co-I,RLMS</td>
</tr>
<tr>
<td>8   δ-3-Carene</td>
<td>1008</td>
<td>1008</td>
<td>t</td>
<td>Co-I,RLMS</td>
</tr>
<tr>
<td>9   α-Terpine</td>
<td>1014</td>
<td>1014</td>
<td>0.1</td>
<td>Co-I,RLMS</td>
</tr>
<tr>
<td>10  p-Cymene</td>
<td>1022</td>
<td>1020</td>
<td>1.4</td>
<td>Co-I,RLMS</td>
</tr>
<tr>
<td>11  Limonene</td>
<td>1024</td>
<td>1025</td>
<td>0.3</td>
<td>Co-I,RLMS</td>
</tr>
<tr>
<td>12  β-Phellandrene</td>
<td>1025</td>
<td>1025</td>
<td>9.2</td>
<td>RLMS</td>
</tr>
<tr>
<td>13  (Z)-β-Ocimene</td>
<td>1037</td>
<td>1032</td>
<td>1.3</td>
<td>Co-I,RLMS</td>
</tr>
<tr>
<td>14  (E)-β-Ocimene</td>
<td>1047</td>
<td>1044</td>
<td>0.1</td>
<td>Co-I,RLMS</td>
</tr>
<tr>
<td>15  γ-Terpine</td>
<td>1055</td>
<td>1054</td>
<td>0.2</td>
<td>Co-I,RLMS</td>
</tr>
<tr>
<td>16  Terpinolene</td>
<td>1086</td>
<td>1086</td>
<td>20.7</td>
<td>Co-I,RLMS</td>
</tr>
<tr>
<td>17  Linalool</td>
<td>1101</td>
<td>1095</td>
<td>t</td>
<td>Co-I,RLMS</td>
</tr>
<tr>
<td>18  1,3,8-p-Menthatriene</td>
<td>1109</td>
<td>1108</td>
<td>t</td>
<td>RLMS</td>
</tr>
<tr>
<td>19  allo-Ocimene</td>
<td>1129</td>
<td>1128</td>
<td>t</td>
<td>Co-I,RLMS</td>
</tr>
<tr>
<td>20  Terpinen-4-ol</td>
<td>1173</td>
<td>1174</td>
<td>0.1</td>
<td>Co-I,RLMS</td>
</tr>
<tr>
<td>21  p-Cymen-8-ol</td>
<td>1183</td>
<td>1179</td>
<td>0.1</td>
<td>RLMS</td>
</tr>
<tr>
<td>22  α-Terpineol</td>
<td>1186</td>
<td>1186</td>
<td>t</td>
<td>Co-I,RLMS</td>
</tr>
<tr>
<td>23  Piperitone</td>
<td>1250</td>
<td>1249</td>
<td>t</td>
<td>RLMS</td>
</tr>
<tr>
<td>24  Piperitenone</td>
<td>1336</td>
<td>1340</td>
<td>t</td>
<td>RLMS</td>
</tr>
<tr>
<td>25  Piperitenone oxide</td>
<td>1362</td>
<td>1366</td>
<td>5.9</td>
<td>RLMS</td>
</tr>
<tr>
<td>26  Germacrene D</td>
<td>1471</td>
<td>1484</td>
<td>t</td>
<td>RLMS</td>
</tr>
<tr>
<td>27  Dill apiole</td>
<td>1621</td>
<td>1620</td>
<td>3.7</td>
<td>RLMS</td>
</tr>
</tbody>
</table>

| Monoterpene hydrocarbons     | 90.1 |
| Oxygenated monoterpens       | 6.1  |
| Sesquiterpene hydrocarbons   | t    |
| Others                       | 3.7  |
| Total                        | 99.9 |

* LRI: Linear retention index on a HP-5MS column; Co-I, Co-elution with authentic standard (Sigma-Aldrich); RI, coherence respect to values reported in ADAMS library; MS, mass spectrum overlapping with those stored in ADAMS, NIST17, and FFNSC3 libraries.

### 3.2. Insecticidal Activity

The *R. segetum* flower EO showed relevant insecticidal activity against the three tested insect species (Table 2). Concerning mosquitoes, the EO showed a considerable efficacy against 3rd instar larvae of the studied species *C. quinquefasciatus*, with a LC$_{50}$ = 27.1 µL L$^{-1}$ and LC$_{90}$ = 42.5 µL L$^{-1}$ (Table 2). These values are lower if compared with those obtained testing many plant EOs as larvicides on the same mosquito species (e.g., *Curcuma zerdoaria* (Christm.) Roscoe EO, LC$_{50}$ = 37.29 µL L$^{-1}$ [64]; *Blumea mollis* (D. Don) Merr. EO, LC$_{50}$ = 71.71 µL L$^{-1}$ [65]; *Zingiber collinsii* Mood & Theilade EO, LC$_{50}$ = 50.11 mg L$^{-1}$ [66]. On the other hand, it should also be noted that—even if several EOs have been found more effective against *C. quinquefasciatus* larvae (e.g., *Lippia berlandieri* Schauer and *Pimpinella anisum* L. oils, both LC$_{50}$ < 7 mg L$^{-1}$ [67])—the LC$_{50}$ value calculated here for the *R. segetum* EO is of interest for further studies. Indeed, according to Pavela [68], EO-based mosquito larvicides achieving LC$_{50}$ values < 50 µL L$^{-1}$ can be considered promising for further development of mosquitocidal products. Further efforts to boost the EO stability and efficacy in the field are still needed [22] to allow its real-world use, with special reference to the encapsulation in nanoemulsions to boost bioactivity and stability in the field [69,70].
Table 2. Lethal concentrations (LC) and lethal doses (LD) of *Ridolfia segetum* flower essential oil against three key insect pests and vectors.

<table>
<thead>
<tr>
<th>Insect Species</th>
<th>Unit</th>
<th>LC/LD₅₀</th>
<th>CIₙ₅</th>
<th>LC/LD₉₀</th>
<th>CIₙ₅</th>
<th>χ² (df = 4)</th>
<th>p-Level</th>
<th>α</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Culex quinquefasciatus</em> 3rd instar larva</td>
<td>µL L⁻¹</td>
<td>27.1</td>
<td>18.9–32.3</td>
<td>42.5</td>
<td>38.9–54.2</td>
<td>0.734</td>
<td>0.574</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td><em>Musca domestica</em> adult female</td>
<td>µg adult⁻¹</td>
<td>50.8</td>
<td>41.2–62.3</td>
<td>147.5</td>
<td>129.5–168.7</td>
<td>3.751</td>
<td>0.289</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td><em>Spodoptera littoralis</em> 3rd instar larva</td>
<td>µg larva⁻¹</td>
<td>37.9</td>
<td>28.5–42.6</td>
<td>99.6</td>
<td>87.2–152.7</td>
<td>2.745</td>
<td>0.615</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td><em>Aedes aegypti</em></td>
<td>µg adult⁻¹</td>
<td>10.5</td>
<td>5.8–15.6</td>
<td>75.2</td>
<td>61.8–93.6</td>
<td>2.485</td>
<td>0.615</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td><em>Aedes albopictus</em></td>
<td>µg adult⁻¹</td>
<td>16.6</td>
<td>11.8–22.2</td>
<td>37.9</td>
<td>28.5–42.6</td>
<td>2.485</td>
<td>0.615</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

CIₙ₅ = 95% confidence interval, df = degrees of freedom; ns = not significant (p > 0.05).

Regarding *M. domestica*, a different toxicity of the *R. segetum* flower EO was found on male and female flies, calculating LD₅₀ values of 10.5 and 50.8 µg adult⁻¹, respectively (LD₉₀ = 75.2 and 147.5 µg adult⁻¹, respectively), with not overlapping 95% confidence intervals (Table 2). Even if a number of promising botanicals have been tested against *M. domestica* larvae, with successful results [71–73], adult flies should also be targeted by proper control action, since they are the vehicle of reproduction and also cause nuisance to humans and livestock, as other muscoid fly species [74,75].

The tested EO showed relatively promising efficacy against *M. domestica*. Based on LC₉₀ values estimated for less sensitive females, we would be able to achieve at least 90% adult mortality approximately with a 15–20% essential oil-based preparation. The different sensitivity between the sexes of *M. domestica* is also confirmed by other studies [52]. In general, females are always less sensitive to chemicals than males. Therefore, in biological tests, it is important to monitor each sex separately, or only females, which are more important in terms of fertility [76,77]. However, even sub-lethal EO concentrations applied to *M. domestica* adults can have a significant impact on the population density of this pest, because they can significantly reduce the fecundity, longevity, and overall vitality of adults as it has been demonstrated for other EOs [52,76,78]. Investigating this phenomenon for the EO of *R. segetum* will be a subject of our further research.

The *R. segetum* flower EO was found significantly toxic to *S. littoralis* 3rd instar larvae, achieving LD₅₀ and LD₉₀ values of 37.9 and 99.6 µg larva⁻¹ (Table 2). Its efficacy seems good against this important pest. Compared to other EOs, the one from *R. segetum* seems to provide average up to superior efficacy when compared to the EO from *Echinophora spinosa* L. (Apiales: Apiaceae) or some EOs obtained from plants of the genus *Thymus* [79,80]. It will also be necessary to determine whether the EO from *R. segetum* causes any phytotoxicity in the treated plants. High concentrations of EOs are known for their potential to exhibit herbicidal activity [81]. However, also it is known that although low doses of plant EOs may not show acute toxicity against *S. littoralis* larvae, they can cause chronic toxicity, as demonstrated, for example, for EOs extracted from *Pimenta racemosa* (Mill.) J.W. Moore, *Origanum vulgare* L., *Salvia sclarea* L., and *Thymus vulgaris* L. Indeed, when these EOs were applied at doses corresponding to the estimated LD₅₀ value, this led to an overall mortality of *S. littoralis* larvae > 70%. In addition, these EOs significantly reduced longevity, fecundity, fertility, and natality of the surviving adults [82]. This phenomenon therefore deserves further studies, including efforts to evaluate the EO of *R. segetum*. It is also important to focus on an appropriate formulation of the botanical insecticide, since the latter can significantly increase the biological efficacy of an EO, thus reducing the needed application concentration. For example, a nanoformulation of the EO from *Mentha spicata* L. (Lamiales: Lamiaceae) increased its efficacy against *Culex pipiens* L. (Diptera: Culicidae) and *M. domestica* by more than 71 and 52%, respectively [70]. The prospects of green micro- and nano-emulsions, increasing the EOs efficacy as well as safety, have also been confirmed by other studies [22,83].

About 80% of the total composition was made up of three constituents, namely α-phellandrene (49.3%), terpinolene (20.7%), and β-phellandrene (9.2%). Thus, it is assumed that the insecticidal effects displayed on the three targets strictly depend on these compounds. α-Phellandrene and terpinolene were proven to be effective as larvicides against *Aedes aegypti* L. and *Ae. albopictus* (Skuse) (Diptera: Culicidae) with LC₅₀ values of 16.6
and 39.9, and 28.4 and 35.6 mg L$^{-1}$, respectively [84]. Terpinolene was also tested by Pavela et al. [85] on C. quinquefasciatus, highlighting an LC$_{50}$ of 25.7 µL L$^{-1}$. β-Phellandrene exhibited contact and fumigant toxicity against the stored-product beetle Sitophilus zeamais (Motschulsky) (Coleoptera: Curculionidae), with LD$_{50}$ and LC$_{50}$ of 2.2 µg adult$^{-1}$ and 3.6 mg L$^{-1}$, respectively [86]. However, even if it should be noted that the majority of components are generally considered to be responsible for biological activity, it is possible that there are synergistic or antagonistic relationships between them, including with minor compounds [87–91]. Further research into this phenomenon is therefore needed.

As for their potential mode of action, evidence from the literature suggests their involvement in cholinergic transmission, leading to an impairment of insect nerve conduction and coordination of neuromuscular system. This can cause an alteration of the insect behavior, lack of motor coordination, and even death [92]. Actually, β-phellandrene has been found to exert inhibition on the acetylcholinesterase activity in the German cockroach, Blattella germanica (L.) (Blattodea: Ectobiidae) [93].

EOs have been often found scarcely toxic to non-target species [17,94,95]. However, in several cases they exhibit relevant toxicity to non-target aquatic species [96,97], pollinators, and biological control agents [98,99]. Therefore, further research assessing the R. segetum EO potential toxicity of non-target species, as well as its long-term effects on physiology and behavior, is needed.

4. Conclusions

In conclusion, the present work explored the insecticidal potential of R. segetum, a Mediterranean species found commonly in cereal fields and uncultivated grounds. GC and GC-MS analyses showed the significant presence of monoterpenes hydrocarbons (90.1%), allowing us to classify the EO in the chemotype dominated by α-phellandrene, terpinolene, and p-cymene. Although developing novel insecticides from the flower EO from R. segetum requires further research, the present study clearly outlines that this traditional food plant provides an effective EO, which can be used for the development and subsequent production of botanical insecticides against highly diverse and harmful insect species. These prospects are also enhanced by the possibility of growing the plant even in arid or rather dry areas [77], an issue that may provide yields similar to those of the analogous habitat and growth of F. vulgare. This potentially alternative crop provides relatively high yields of EO (about 1%), and is both a food and medicinal plant, therefore applications of botanical insecticides based on the EO from R. segetum does not give rise to any concerns.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11040304/s1, Table S1: Current knowledge about the main compounds (≥2%) detected in the essential oils extracted from different plant parts of Ridolfia segetum.


Funding: This work was supported by grant from MIUR-ITALY PRIN 2017 (Project N. 2017A95NCJ) (M. Bruno). R. Pavela would like to thank the Ministry of Agriculture of the Czech Republic for its financial support concerning botanical pesticide and basic substances research (Project MZE-RO0418).

Acknowledgments: We are grateful to N. Ntalli for inviting this article as part of her Agriculture Special Issue “Biopesticides: The Naturally Originating Plant Protection Products and Biocides”.

Conflicts of Interest: The authors declare no conflict of interest.
References


18. Jankowska, M.; Rogalska, J.; Wyszkowska, J.; Stankiewicz, M. Molecular targets for components of essential oils in the insect nervous system—A review. *Molecules* 2017, 23, 34. [CrossRef]


37. Pavela, R. Lethal and sublethal effects of thyme oil (*Thymus vulgaris* L., Lamiaceae) against *Culex quinquefasciatus Say* and *Spodoptera littorialis* (Boisd.). *Ind. Crops Prod.* 2017, 105, 603–610. [CrossRef]


42. Wilke, A.B.B.; Beier, J.C.; Benelli, G. Filariaasis vector control down-played due to the belief the drugs will be enough—Not true! *Entomol. Gen.* 2020, 40, 15–24. [CrossRef]


50. Pavela, R. Insecticidal properties of *Pimpinella anisum* essential oils against the *Culex quinquefasciatus* and the non-target organism *Daphnia magna*. *J. Asia Pac. Entomol.* 2014, 17, 287–293. [CrossRef]


52. Pavela, R. Acute, synergistic and antagonistic effects of some aromatic compounds on the *Spodoptera littoralis* Boisd. (Lep., *Noctuidae*) larvae. *Ind. Crops Prod.* 2014, 60, 247–258. [CrossRef]


64. Sutthanont, N.; Attrapadung, S.; Nuchprayoon, S. Larvicidal activity of synthesized silver nanoparticles from *Curcuma zedoaria* essential oil against *Culex quinquefasciatus*. *Insects* 2019, 10, 27. [CrossRef]


90. Liu, T.T.; Chao, L.K.P.; Hong, K.S.; Huang, Y.J.; Yang, T.S. Composition and insecticidal activity of essential oil of *Bacopa caroliniana* and interactive effects of individual compounds on the activity. *Insects* 2020, 11, 23. [CrossRef] [PubMed]


