Acute and sub-lethal toxicity of eight essential oils of commercial interest against the filariasis mosquito *Culex quinquefasciatus* and the housefly *Musca domestica*

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Abstract

The massive use of synthetic insecticides led to negative effects on the environment and human health. Therefore, researchers looked at natural products as effective alternatives to conventional pesticides. Here, commercially valuable essential oils (EOs) were selected from mint (*Mentha* x *piperita*, *Mentha spicata*), basil (*Ocimum basilicum*), helichrysum (*Helichrysum italicum*), yarrow (*Achillea ligustica*), geranium (*Pelargonium odoratissimum*), cinnamon (*Cinnamomum verum*) and ginger grass (*Lippia alba*). The chemical composition of these EOs assayed was analyzed by GC-MS. Then, we investigated their insecticidal potential in acute and sub-lethal toxicity assays against mosquito vectors of filariasis (*Culex quinquefasciatus*) and house flies (*Musca domestica*). Against *C. quinquefasciatus* 4th instar larvae, the most toxic EO was *C. verum* (LC_{50} = 40.7 µl L^{-1}), followed by *L. alba* (LC_{50} = 59.6 µl L^{-1}), while against *M. domestica* adults, the most toxic EOs were *C. verum* and *H. italicum* (LD_{50} = 42 µg $adult^{-1}$). The exposure of mosquito larvae to a sub-lethal concentration (LC_{30}=25 mg L^{-1}) led to a reduction of adult emergence and fertility. Besides, adult flies that survived after exposure to a sub-lethal dose of *C. verum* EO (LD_{20}=10 µg $adult^{-1}$) showed a marked decrease in male and female longevity, as well as to a reduction in fecundity, fertility, and natality. Overall, *C. verum* and *H. italicum* EOs showed a highly promising insecticidal potential on two key insect vectors and pests. The relatively low prices of the selected EOs, their availability on the market and the noteworthy global production of the bulky materials, make them as ideal candidate ingredients to be used in insecticidal formulations.

Keywords: *Achillea ligustica; Cinnamomum verum; Helichrysum italicum; Lippia alba; Ocimum basilicum; Pelargonium odoratissimum*

46 **1. Introduction**

47

48 The massive use of synthetic insecticides until the second half of 20th century
49 produced negative effects on the environment and human health, and forced the agrochemical
50 companies to reduce and/or avoid the use of a substantial number of detrimental substances
51 from their chemical arsenals. At the same time, researchers looked at natural products as
52 effective alternatives to conventional insecticides (Pavela and Benelli, 2016). As a matter of
53 fact, it has been estimated that the market of plant-borne insecticides will reach the 7 % of the
54 global pesticide market by 2025 (Isman, 2015) and that of synthetic pesticides is expected to
55 decline by 1.5 % per year (Thakore, 2006). Among natural products, plant essential oils (EOs)
56 are complex mixtures (even above hundreds of constituents) of small, volatile and lipophilic
57 compounds produced by several aromatic species. They gained commercial importance
58 because of their massive use in flavors and fragrances, foodstuffs, beverages, cosmetics and
59 pharmaceutics (Lubbe and Verpoorte, 2011). Currently, 300 EOs are used worldwide on an
60 industrial scale (CBI, 2009). They are obtained from several botanical families among which
61 Asteraceae, Lamiaceae, Lauraceae, Geraniaceae and Verbenaceae are currently recognized as
62 the most important ones (Benelli 2015a; Pavela, 2016).
63 Recently, the European Food Safety Authority (EFSA) is simplifying the regulatory
64 path for some botanicals, including EOs, by evaluating them as 'low-risk active substances'
65 (LRASs) as reported in the EC Regulation No. 1107/2009. Important strengths favoring the
66 application of EO-based insecticides are the availability of bulky materials from which some
67 EOs are obtained, the high yields of EOs obtainable from cheap plant sources, the relatively
68 ease of preparation by distillation and chemical characterization by gas chromatography
69 coupled with mass spectrometry (GC-MS) (Rubiolo et al., 2010), and their acknowledged
70 safety to humans and environment (they are Generally Recognized as Safe, GRAS) (Isman et

al., 2011). Actually, the restricted regulatory requirements are limiting the diffusion and marketing of EOs-based insecticides (Pavela and Benelli, 2016).

In the present work, we investigated the insecticidal effects of an EOs panel of commercial interest against two insects of high economic importance, the filariasis mosquito vector *Culex quinquefasciatus* Say (Diptera: Culicidae), which is also an important vector of St. Louis encephalitis and West Nile virus (Benelli, 2015b; Vadivalagan et al., 2017), and the housefly *Musca domestica* L. (Diptera: Muscidae), which transmit pathogens of public health relevance causing more than one hundred diseases (WHO, 1991). The needing of novel and effective green pesticides to control these two insects is pressing (Benelli and Mehlhorn, 2016; Benelli et al., 2016; Benelli and Romano, 2017), since both developed resistance to a rather wide number of synthetic pesticides currently marketed (Hardstone et al., 2014; Naqqash et al., 2016; Benelli and Beier, 2017).

For the purpose, commercially valuable EOs were selected from mint (*Mentha* x *piperita* L., *Mentha spicata* L.), basil (*Ocimum basilicum* L.), helichrysum (*Helichrysum italicum* (Roth) G. Don), yarrow (*Achillea ligustica* All.), geranium (*Pelargonium odoratissimum* (L.) L'Hér.), cinnamon (*Cinnamomum verum* J. Presl) and ginger grass (*Lippia alba* (Mill.) N.E.Br. ex Britton & P. Wilson) (Fig. 1). The commercial values of these EOs have been estimated as 155-230, 80-100, 40-45, 30-45 and 18-20 €/kg for *C. verum*, *P. odoratissimum*, *O. basilicum*, *M.* x *piperita* amd *M. spicata*, respectively (Lubbe and Verpoorte, 2011). The relatively low prices, their availability on the market and the noteworthy global production of the bulky materials, make them as ideal candidate ingredients to be used in insecticidal formulations, also in organic farms.

Mint EOs, like those coming from *M.* x *piperita* and *M. spicata*, are dominated by oxygenated monoterpenes such as menthol, carvone, pulegone and piperitone. They are used against skin irritations and sunburn, as well as antipyretic, anti-inflammatory, and nasal

96 decongestant. Other applications consist in their incorporation in perfumes and as flavoring
97 agents in foodstuffs (Kumar et al. 2011). Basil EO is recognized as antioxidant, anesthetic,
98 anti-inflammatory, antimicrobial and antiproliferative agent. These properties are ascribable
99 to the presence of methyl chavicol and linalool (Rodrigues et al., 2017; Varga et al., 2017).
100 The economic interest around the helichrysum EO is linked to the abundance of the
101 monoterpenoid neryl acetate which makes it an ideal ingredient of glamorous perfumes and
102 personal care products (Appendino et al., 2015). Helichrysum EO is also effective as wound
103 healing agent and against several skin disorders (Schnaubelt, 1999). Ligurian yarrow EO has
104 exhibited important inhibitory properties against oral pathogens (Cecchini et al., 2012), while
105 apple-scented geranium EO obtained from *P. odoratissimum* is used in perfumery and
106 cosmetics, as well as in aromatherapy and the food industry (Lis-Balchin and Roth, 2000).
107 Frequently it is used to replace the very expensive EO from *Rosa* x *damascena* Herrm., which
108 account up to $7500/kg (Blerot et al., 2016). Cinnamon EO is widely used on an industrial
109 level, e.g., to prepare pharmaceutics, seasonings, cosmetics, food and beverages (Li et al.,
110 2013). The major component of this oil is the aromatic cinnamaldehyde, which is responsible
111 for important biological effects, namely anti-inflammatory (Chao et al., 2005), antioxidant
112 (Murcia et al., 2004) and antibacterial ones (Chang et al., 2001). The ginger grass EO
113 obtained from *L. alba* is valuable for the pharmaceutical industry due to the diverse biological
114 activities exhibited, namely antioxidant, antimicrobial, anti-inflammatory, sedative,
115 antigenotoxic, immunomodulatory and antiproliferative (García et al., 2017). Its composition
116 is quite variable, being characterized by different chemotypes according to genetic factors and
117 geographic origin (Hennebelle et al., 2008). Among these, the carvone/limonene chemotype is
118 considered as the most important in South America (da Silva Lima et al., 2016; U.N., 2005).
119 The effects of EOs against insect pests and vectors can be classified into two main
120 groups, namely behavioral (e.g., repellent, anti-feedant, inhibition of oviposition) and

121 physiological ones (e.g., acute and sub-lethal toxicity, inhibition of development and growth)
122 (Isman, 2018). In the present work, after analyzing the chemical composition of the above
123 mentioned EOs by gas chromatography-mass spectrometry (GC-MS), we assayed them for
124 acute toxicity against 4th instar larvae of *C. quinquefasciatus* and adults of *M. domestica*.
125 Once the most effective EO was determined, we evaluated the effects of its sub-lethal
126 concentrations on egg emergence, longevity and fertility in both targeted insects.
127
128 **2. Materials and Methods**
129
130 2.1. Essential oils
131
132 *C. verum*, *M. crispa, O. basilicum* and *M. piperita* EOs were kindly provided by
133 Phalada Agro Research Foundations Pvt Ltd. (http://phaladaagro.com), Bangalore, India. The
134 EO of *L. alba* was purchased from Centro de Investigación de Excelencia – CENIVAM
135 (http://cenivam.uis.edu.co/cenivam/), Bucaramanga, Santander, Colombia. EOs of *H. italicum*
136 and *P. odoratissimum* were kindly furnished by APPO (Associazione Produttori Piante
137 Officinali delle Marche, http://www.ladistilleriaappo.it), Ancona, Italy. *A. ligustica* EO was
138 obtained from the aerial parts collected from a population cultivated in the Botanical Garden
139 of the University of Camerino in June 2016 (Cecchini et al., 2012; Maggi et al., 2009). For
140 this sample, a voucher specimen was archived in the *Herbarium Camerinensis* of the School
141 of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy, under the
142 codex CAME 13420.
143
144 2.2. Chemical analysis of the EOs by GC-MS
145

146 Chemical analysis of EOs was performed by an Agilent 6890N gas chromatograph
147 coupled to a 5973N mass spectrometer and equipped with a HP-5 MS (5%
148 phenylmethylpolysiloxane, 30 m, 0.25 mm i.d., 0.1 μm film thickness; J & W Scientific,
149 Folsom) capillary column. The analytical conditions used were as follows: oven programmed
150 for 5 min at 60°C then 4°C min^{-1} up to 220°C, then 11°C min^{-1} up to 280°C, held for 15 min;
151 temperatures of injector and detector were set to 280 °C; He was used as the carrier gas with a
152 flow rate of 1 mL min^{-1}; split ratio was1:50; mass spectra were acquired in full scan in the
153 range of 29–400 $m\ z^{-1}$ using electron-impact (EI, 70 eV) mode. For each EO, a dilution in *n*-
154 hexane (1:100) was prepared and 2 μL of the solution was injected into the GC-MS system.
155 For data analysis, the MSD ChemStation software (Agilent, Version G1701DA D.01.00) and
156 NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library v. 2.0
157 were used. The identification of peaks was made by means of co-injection with standards
158 available in our laboratory, together with correspondence of retention indices (according to
159 Van den Dool and Kratz formula) and mass spectra with respect to those occurring in
160 ADAMS, NIST 08 and FFNSC2 libraries (Adams, 2007, NIST 08, 2008, FFNSC2, 2012).
161 Semi-quantification of essential oil components was made by peak area normalization
162 considering the same response factor for all volatile components. Values (%) were the mean
163 of 3 chromatographic analyses.
164
165 2.3. Insects
166
167 *C. quinquefasciatus* was reared in the laboratory colony of Crop Research Institute,
168 Czech Republic. The larvae were fed on dog biscuits and yeast powder in the ratio 3:1. Adults
169 were provided with a sucrose solution (10% w:v) and for blood feeding were a 1-week-old
170 chick. Early 4th instar *C. quinquefasciatu*s larvae were used in the study.

171 Adult houseflies (*Musca domestica* L.) were tested here. They were obtained from a
172 laboratory colony of Crop Research Institute, Prague. Larvae were reared in the mixture of
173 sterilized bran with milk powder and water. Adults were fed ad libitum with sugar water
174 (10% w:v) and to milk powder.
175 All tested insects were maintained at 25°C ± 1°C, with 50–70% RH, and 16:8
176 photoperiod (L:D). All experiments were performed under the same conditions.
177
178 2.4. Larvicidal toxicity on mosquitoes
179
180 Mosquito larvicidal trials were carried out according to WHO (1996) standard
181 procedures, with slight modifications (Pavela, 2015b). The essential oils were diluted in
182 dimethyl sulfoxide to prepare a serial dilution of the test dosage. For experimental treatment,
183 1 mL of serial dilution was added to 224 mL of distilled water in a 500-mL glass bowl and
184 shaken gently to produce a homogeneous test solution. The larvae of *C. quinquefasciatus*
185 were transferred in water into a bowl of the prepared test solution (25 larvae/beaker). Four
186 duplicate trials were carried out for every sample concentration, and for each trial, a negative
187 control was included using distilled water containing the same amount of dimethyl sulfoxide
188 as the test sample. A different series of concentrations (resulting from the previous screening)
189 was used for each essential oil to obtain mortality ranging between 10% and 90%. At least 5
190 concentrations were selected for the calculation of lethal doses. Mortality was determined
191 after 24 h of exposure, during which no food was given to the larvae.
192
193 2.5. Adulticidal toxicity on house flies
194

195 Acute toxicity (measured as mortality after 24 h) was determined by topical
196 application on *M. domestica* adults (3–6 days old). A micro-electric applicator was used to
197 deliver 1 μL doses of different dosages of EOs mixed with acetone, to the pronota of CO_2-
198 anesthetized flies. Initial screening to approximate the active dose range determined a range
199 of doses that were used to establish the lethal doses. Certified acetone was used as the control
200 treatment. A minimum of 5 concentrations were replicated at least for 4 times (80 flies per
201 single replication) in the final bioassays. All treated flies from each replicate were placed in a
202 plastic box (10 cm diameter x 12 cm high) and had free access to water. Mortality was
203 assessed 24 h after the treatment was performed. Flies that did not respond to mechanical
204 stimulation were considered dead.
205
206 2.6. Sub-lethal effects on mosquitoes
207
208 At the beginning of the fourth instar, *C. quinquefasciatus* larvae were moved into a
209 plastic container (20 x 20 x 20 cm) with 3 L of drinking water. After acclimatization (after
210 approximately 1 h), a dose of the EO found as most effective in acute toxicity assays (*C.*
211 *verum*) was mixed into the water, corresponding to the calculated LC_{30} (25 mg L^{-1}). The EO
212 was emulsified using DMSO; water with an adequate DMSO content was used as control. 100
213 larvae were stored in each container. After 12 h of exposure, the larvae were transferred into
214 clean water, where they were left until the incubation of adults. The larvae were fed on dog
215 biscuits and yeast powder in a 3:1 ratio. Larvae mortality was determined after 24 and 48 h of
216 exposure; total mortality, percentage of incubated adults, and their sex were determined.
217 The survived adults were used for determination the effect of *C. verum* EO on fertility.
218 The mosquitoes were kept on breeding cages (25 x 25 x 30 cm). As food sugar water was
219 given and to lay their eggs, a plastic container with water was placed in the middle of each

220 cage. Untreated mosquitoes were used as control. The number of laid eggs per single female
221 mosquito was noted each day. To evaluate emergence and for calculation of potential number
222 of larvae on all survive female, 100 freshly-laid eggs were analyzed. The eggs were placed
223 into plastic dishes (diameter of 12cm and height of 6 cm) filled with water. The number of
224 emerged larvae were noted.
225 All tests were replicated 3 times, with the conditions of the experiment as described in
226 "Insects" paragraph.
227
228 2.7. Sub-lethal effects on house flies
229
230 The following method was used to evaluate the sub-lethal effects of *C. verum* EO on
231 longevity, fecundity and natality of *M. domestica*. Housefly adults (1-2 days old) were
232 divided onto their sex. The *C. verum* oil was applied topically on fly adults at its LD_{20} dose
233 (10 μg fly^{-1}). The EO application was done following the same method described in the
234 paragraph "Adulticidal toxicity on house flies". To shed light on the effects of *C. verum* EO
235 on longevity and fecundity, *M. domestica* flies were kept on breeding cages (25 x 25 x 30
236 cm). Into each cage there, 20 male and 20 female flies were placed. As food were given sugar
237 water and powdered milk and to lay their eggs, a Petri dish was placed in the middle of each
238 cage, arranged with cotton which was soaked in sweet milk. Untreated males and females
239 were used as control.
240 The mortality of both sexes and the number of laid eggs per single female fly were
241 supervised each day. The ratio of laid eggs per female fly was regulated by the number of
242 female flies present in each assay. To estimate fly aging post-treatment, a period, within
243 which 50% and 99% mortality (LT_{50} and LT_{90}) of the fly adults was seen, was set, with use of
244 the linear regression analysis.

To evaluate natality, we considered 100 freshly-laid eggs, i.e. on each day within the entire period of 10 days (total n=1000) in which the test was performed. The eggs were then placed into plastic dishes (diameter of 12 cm and height of 6 cm) where feedings were arranged. The, the number of emerged larvae were evaluated.

All tests was replicated 3 times, with the conditions of the experiment as described in "Insects" paragraph.

2.8. Statistical analysis

Experimental tests showing > 20 % of control mortality was discharged and repeated. When the control mortality ranged from 1–20%, the observed mortality was corrected by the Abbott's formula (Abbott, 1925) the LC_{50}, LC_{90} regression equation, and a 95% confidence limit were calculated by probit analysis (Finney, 1971).

Lethal time was calculated as time (in days) to indigent of 50% or 99% natural mortality of adult *M. domestica*. 95% Confidence intervals (CI) were not adjusted for multiple inferences. Essential oil activity was considered significantly different when the 95% CI fail to overlap.

Data about average number of eggs, mortality, fertility and natality were statistically evaluated by using a two-fold F-test (* p=0.05; ** p=0.01). Mortality (%) was sujected to angular transformation (y = arcsen x √%) before ANOVA.

3. Results and Discussion

3.1. Chemical composition of the eight essential oils

The chemical compositions of the commercial EOs assayed for insecticidal activity are reported in Table. 1, whereas the GC-MS chromatograms of the most active ones are depicted in Fig. 2. A total of sixty-six volatile components was identified in the EO of *M. x piperita*, corresponding to 99.8% of the total composition. This EO was dominated by oxygenated monoterpenes (81.0%) such as menthol (26.0%), menthone (20.7%), *iso*-menthone (11.6%), menthyl acetate (9.5%) and *neo*-menthol (5.3%) (Fig. 3). Monoterpene hydrocarbons gave a minor contribution (12.3%), with limonene as the most representative compound (7.1%). This composition is in line with peppermint batches of industrial interest (Fejér et al., 2017).

A total of fifty-five compounds was identified in the EO of *M. spicata*, accounting for 99.4% of the total EO. Also in this case, the major fraction of EO was given by oxygenated monoterpenes (66.7%), but monoterpene hydrocarbons were here more abundant (27.8%) compared with peppermint. Carvone (58.2%) and limonene (22.5%) (Fig. 3) strongly characterized the *M. spicata* EO. This chemical profile was consistent with those reported in literature (Chrysargyris et al., 2017).

The EO of *O. basilicum* was composed of thirty-seven components, accounting for 99.8% of the total composition. The EO was strongly characterized by only two components, namely methyl chavicol (77.9%) and linalool (15.6%) (Fig. 3). Based on these results we can assign this basil EO sample to the high-methyl chavicol chemotype as proposed by Varga et al., (2017).

A total of fifty-four components was identified in the EO of *H. italicum*, accounting for 95.2% of the overall composition. The EO was characterized by oxygenated monoterpenes (57.8%) such as the ester neryl acetate (45.4) which is responsible for the characteristic floral scent (Fig. 3). Other noteworthy components in this fraction were neryl propanoate (5.0%) and linalool (3.9%). In addition, the sesquiterpene hydrocarbons (27.8%) gave an important contribution to the overall EO composition. Among them, γ-curcumene (9.0%) and *ar*-

295 curcumene (7.6%) were the most abundant compounds. Finally, oxygenated sesquiterpenes
296 (6.2%) and monoterpene hydrocarbons (2.3%) were poor, being mainly represented by
297 rosifoliol (3.7%) and α-pinene (2.3%), respectively. The overall chemical profile of this EO
298 was in line with those found in other spontaneous and cultivated populations of *H. italicum*
299 used on an industrial level (Morone-Fortunato et al., 2010; Melito et al., 2016; Leonardi et al.,
300 2013).

301 A total of one hundred and thirty-two volatile components was identified in the EO
302 from the aerial parts of *A. ligustica*, corresponding to 88.5% of the total composition. This EO
303 was characterized by oxygenated monoterpenes (29.1%), sesquiterpene hydrocarbons
304 (23.7%), oxygenated sesquiterpenes (22.3%) and monoterpene hydrocarbons (12.3%), with
305 linalool (10.8%), germacrene D (11.8%), viridiflorol (12.6%) and β-pinene (6.0%) as the
306 most representative compounds, respectively (Fig. 3). Other components occurring in
307 noteworthy levels (> 2%) were terpinen-4-ol (6.0%), 1,8-cineole (4.4%) and (*E*)-
308 caryophyllene (2.1%). This profile was consistent with that previously reported by us
309 (Cecchini et al., 2012; Maggi et al., 2009) and showed some differences with respect to
310 batches of different geographic origin (Tuberoso et al., 2005; Badeer et al., 2007).

311 A total of ninety-two compounds was identified in the EO of *P. odoratissimum*,
312 accounting for 98.5% of the total EO. The chemical composition of this EO was dominated
313 by oxygenated monoterpenes (71.5%) such as citronellol (30.1%), *iso*-menthone (16.2%) and
314 citronellyl formate (9.1%) (Fig. 3). Minor contributions were given by sesquiterpene
315 hydrocarbons (14.6%) and monoterpene hydrocarbons (7.5%), with 6,9-guaiediene (5.7%)
316 and α-pinene (2.9%) as the most representative compounds, respectively. The chemical
317 profile of this EO showed some similarities with that studied by Matusinsky et al. (2015) who
318 found citronellol (24.9%), geraniol (13.0%), citronellyl formate (7.7%) and *iso*-menthone
319 (4.7%) as the major compounds. On the other hand, deep differences emerged when

320 compared with investigations of Balchin and Roth (2000) and Andrade et al. (2011) who
321 found methyl eugenol (31.2-79.8%) and *iso*-menthone (4.6-16.9%), and methyl eugenol
322 (96.8%) as the main volatile components, respectively.

323 A total of thirty-nine components was identified in the EO from the bark of *C. verum*,
324 accounting for 99.1% of the total composition. They were almost entirely represented by
325 aromatic compounds (97.8%), with (*E*)-cinnamaldehyde (82.7%) and (E)-*o*-methoxy
326 cinnamaldehyde (10.1%) as the most representative compounds (Fig. 3). The remaining
327 components were all present in scant amounts (≤0.8%). Interestingly, this EO was almost
328 devoid of eugenol (occurring at trace levels), which is often found as a major volatile
329 component of the cinnamon bark (Azeredo et al., 2014; Yap et al., 2015; Jumbo et al., 2014;
330 Ju et al., 2018). This chemical profile was in line with those reported in literature for
331 cinnamon bark EO (Li et al., 2013; Sienkiewicz et al., 2014).

332 A total of ninety-three compounds was identified in the EO of *L. alba*, corresponding
333 to 99.2% of the total composition. The EO was characterized by oxygenated monoterpenes
334 (42.8%), monoterpene hydrocarbons (32.9%) and sesquiterpene hydrocarbons (21.9%), with
335 carvone (35.2%), limonene (32.0%) and germacrene D (14.8%) as the major components,
336 respectively (Fig. 3). The remaining constituents were all present in scant amounts, with
337 piperitenone (2.1%), β-bourbonene (1.8%), piperitone (1.1%) and β-elemene (1.0%) as the
338 most abundant ones. According to Hennebelle et al. (2008), *L. alba* EOs show a broad
339 chemical polymorphism, with eight different chemotypes and the EO investigated in this
340 study belonged to the limonene/carvone chemotype.

341

342 3.2. Acute and sub-lethal toxicity of the essential oils against mosquitoes and flies

343

344 Results of the acute toxicity experiments on *C. quinquefasciatus* and *M. domestica* of
345 the eight tested EOs were provided in Tables 2 and 3, respectively. Against 4th instar larvae of
346 *C. quinquefasciatus* mosquitoes, the most toxic EO was *C. verum* (LC_{50} = 40.7 µl L^{-1}),
347 followed by *L. alba* (LC_{50} = 59.6 µl L^{-1}), *O. basilicum* (LC_{50} = 68.6 µl L^{-1}), *M. spicata* (LC_{50}
348 = 88.2 µl L^{-1}) and *A. ligustica* (LC_{50} = 89.5 µl L^{-1}), while the other three tested EOs showed
349 LC_{50} values higher than 100 µl L^{-1} (Table 2).
350 Furthermore, against adults of *M. domestica* flies, the most toxic EOs were *C. verum*
351 and *H. italicum* (LD_{50} = 42 µg $adult^{-1}$), followed by *P. odoratissimum* (LD_{50} = 54 µg $adult^{-1}$),
352 *Mentha* x *piperita* (LD_{50} = 59 µg $adult^{-1}$), *O. basilicum* (LD_{50} = 70 µg $adult^{-1}$) and *M. spicata*
353 (LD_{50} = 86 µl L^{-1}), while the other two tested EOs showed LD_{50} values higher than 100
354 µg/adult (Table 3).
355 Since *C. verum* showed the best toxic potential against both targeted insect species, we
356 selected it for sub-lethal tests. Table 4 showed the emergence and fertility of *C.*
357 *quinquefasciatus* adults that survived to a sub-lethal dose (LC_{30}=25 mg/l) of *C. verum* EO
358 administered to 4th instar larvae. The exposure to a sub-lethal concentration of the selected EO
359 led to a significant larval mortality as well as reduction in adult emergence. The fertility of
360 adults, in terms of both eggs/female and emergence from eggs was also reduced (Table 4). In
361 addition, Table 5 summarized the longevity and fertility of *M. domestica* adults that survived
362 post-exposure to a sub-lethal dose (LD_{20}=10 µg $adult^{-1}$) of *C. verum* EO. The EO LD_{20}
363 treatment on flies led to a marked decrease in male and female longevity, as well as to a
364 significant reduction in fecundity, fertility and natality (Table 5).
365 As far as we know, this is the first report on the effect of lethal and sub-lethal doses or
366 concentrations of the EO from *C. verum* on insects. As demonstrated by our tests, although
367 the efficacy of lower than lethal doses or concentrations of this EO does not cause acute
368 toxicity, it results in subsequent significant reduction of longevity, fecundity and fertility of

369 *M. domestica* and *C. quinquefasciatus* adults. If compared with the great amount of data
370 available about acute toxicity of EOs against insects (Benelli 2015b; Pavela, 2015a), studies
371 testing their sub-lethal toxicity are limited. In agreement with our data, a sub-lethal dose of
372 the EO from *Thymus vulgaris* L. was previously found to reduce not only the fecundity of the
373 treated *M. domestica* adults, but also the vitality of larvae hatched from eggs laid by the
374 treated females, resulting in almost 100% mortality of the larvae (Pavela, 2007). A similar
375 negative effect of the thyme EO was also found when applied in lethal concentrations against
376 *C. quinquefasciatus* larvae (Pavela, 2009), where even a short-term exposure caused
377 subsequent almost 90% mortality of the larvae. The overall significant reduction of larval
378 natality and of the fecundity of subsequently hatched adults of *C. quinquefasciatus* was also
379 observed for furanochromene isolated from the seeds of *Ammi visnaga* (Pavela et al., 2016).
380 Thus, as repeatedly demonstrated, even sub-lethal doses or concentrations of some secondary
381 plant metabolites may have a significant negative impact on the fertility and vitality of the
382 subsequent generation. This finding is very important as potential botanical insecticides
383 developed based on these EOs may result in a significant reduction of the overall frequency of
384 incidence of the target insect species even when applied in lower doses or concentrations,
385 thereby significantly reducing the risk of disease transmission.
386 Although the effects of several EOs against *M. domestica* adults were studied in the
387 past (Pavela, 2008; Zhang et al., 2017), only sporadic information about the efficacy of the
388 EO from *C. verum* is available. The effects of EOs against mosquito larvae have been studied
389 to a much higher extent (Pavela, 2015a). Currently, EOs showing $LC_{90} < 100$ µl L^{-1} in tests
390 are considered as highly promising for the development of botanical larvicides. In our case,
391 this efficacy was reached only by the EO from *C. verum,* with LC_{90} estimated as 64 µl L^{-1}.
392 Other authors have also observed a very good larvicidal efficacy of EOs obtained from

393 *Cinnamomum* genus. For example, Cheng et al., (2004) estimated LD_{90} as 79 µl L^{-1} on larvae
394 of *Aedes aegypti* L. for the EO from leaves of *Cinnamomum osmophloeum* Kaneh.
395 Overall, the relevant insecticidal activity exhibited by the cinnamon EO seems to be
396 linked to the major compound, i.e. cinnamaldehyde (Fig. 3). This aromatic aldehyde has
397 shown a wide spectrum of efficacy, being toxic on several arthropod vectors (Jeon et al.,
398 2017; Tak and Isman, 2017). At cellular level, cinnamaldehyde is able to inhibit enzymes
399 involved in cytokinesis, as well as to reduce the ATPase activity of cell membranes (Gill and
400 Holley, 2006). Furthermore, it causes loss of membrane integrity and membrane
401 depolarization and decrease cell respiration (Bouhdid et al., 2010). Cinnamon EO is
402 recognized as safe by the United States Food and Drug Administration (FDA) (Jeon et al.,
403 2017). Thus, its use as ingredient in botanical insecticides is highly recommended.
404
405 **4. Conclusions**
406
407 Overall, based on our findings, the *C. verum* and *H. italicum* EOs showed a highly
408 promising insecticidal potential on two key insect pests and vectors. The relatively low prices
409 of the selected EOs, their availability on the market and the noteworthy global production of
410 the bulky materials, make them as ideal candidate ingredients to be used in insecticidal
411 formulations.
412 Considering that we found a significant effect of sub-lethal doses or concentrations of
413 the EO from *C. verum* on the target insect species, further tests will be needed to ascertain the
414 effect of this EO on non-target organisms, although as already demonstrated several times,
415 EOs are relatively friendly to non-target organisms including humans (Pavela, 2016; Pavela
416 and Benelli, 2016), aquatic plankton (Pavela, 2014) or fishes (Pavela and Govindarajan,
417 2017). Given that as previously found, synergistic and antagonistic relationships play an

important role in insecticidal efficacy of EOs (Pavela, 2015b; Benelli et al., 2017a,b), it will be important to study mutual synergistic relationships between the major compound – cinnamaldehyde and other minor compounds to understand these relationships and to try to experimentally increase the insecticidal efficacy.

Besides, further optimization and standardization of their insecticidal activity through microencapsulation (Pavela, 2016) and formulation on green-coated nanomaterials (Benelli 2016, 2018) are ongoing.

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Table

Table 1. Chemical composition of the eight essential oils assayed for insecticidal activity.

Component[a]	RI[b]	RI lit[c]	Lamiaceae			Asteraceae		Geraniaceae	Lauraceae	Verbenaceae	ID[e]
			M. x *piperita*	*M. spicata*	*O. basilicum*	*H. italicum*	*A. ligustica*	*P. odoratissimum*	*C. verum*	*L. alba*	
4-methyl-pentanol	838	830						0.1			b,c
(2*E*)-hexenal	849	846								Tr[f]	b,c
(3E)-hexenol	850	844	0.1					0.1			b,c
(3Z)-hexenol	850	850					Tr				b,c
n-hexanol	863	863					Tr				b,c
2-methyl butyl acetate	876	875								Tr	b,c
1-nonene	888	888					Tr				b,c
styrene	897	897							0.1		b,c
2,5-diethyl-tetrahydrofuran	899	896	0.1	0.1							b,c
isobutyl isobutyrate	912	911					Tr			Tr	b,c
2-butenoic acid, 3-methyl-, ethyl ester	920	924								Tr	b,c
α-thujene	921	924		0.1			Tr			Tr	b,c
α-pinene	926	932	1.5	0.9		2.0	0.5	3.0	0.1	Tr	a,b,c
ethyl tiglate	934	929								Tr	b,c
camphene	939	946					Tr		0.1	0.1	a,b,c
thuja-2,4(10)-diene	945	953					Tr				b,c
benzaldehyde	954	952					Tr		0.7	Tr	a,b,c
sabinene	966	969	0.7	0.6			0.8			Tr	a,b,c
β-pinene	969	974	1.7	1			6.0			Tr	a,b,c
1-octen-3-ol	976	974					Tr			Tr	a,b,c
6-methyl-5-hepten-2-one	985	981			0.1						b,c
3-octanone	986	979								Tr	b,c
myrcene	989	988	0.8	1.6			Tr	0.3		0.4	a,b,c
3-octanol	998	988	0.8	0.4						Tr	b,c
α-phellandrene	1002	1002					Tr		1.2		a,b,c
isobutyl 2-methylbutyrate	1004	1004					Tr				b,c
(3Z)-hexenyl acetate	1009	1004					Tr				b,c
α-terpinene	1014	1014		0.1			0.9				b,c
1,4-cineole	1016	1012					Tr				b,c

2-methylbutyl isobutyrate	1018	1021								Tr	b,c
p-methyl-anisole	1018	1015				0.2					b,c
p-cymene	1023	1020	0.2	0.6		tr	1.6	1.2	Tr	Tr	a,b,c
limonene	1025	1024	7.1	22.5	0.1	0.3		0.7	Tr	32.0	a,b,c
β-phellandrene	1025	1025					0.2				b,c
1,8-cineole	1026	1026	0.5		0.3		4.4	0.3			a,b,c
(*Z*)-β-ocimene	1038	1032					Tr	0.8		Tr	a,b,c
salicylaldehyde	1046	1044							0.1		b,c
(*E*)-β-ocimene	1047	1044					Tr	0.1		0.2	a,b,c
isobutyl angelate	1049	1045					Tr				b,c
γ-terpinene	1056	1054		0.2			1.8	0.1		Tr	a,b,c
cis-sabinene hydrate	1063	1065		0.3			0.1				b,c
cis-linalool oxide	1070	1067			0.1			0.1			b,c
acetophenone	1070	1073							Tr		b,c
n-octanol	1072	1063	0.1				Tr				b,c
terpinolene	1085	1086	0.1	0.1			0.4			Tr	a,b,c
p-cymenene	1086	1089								Tr	b,c
trans-linalool oxide	1087	1084			0.1						b,c
6-camphenone	1090	1095					Tr				b,c
o-guaiacol	1091	1089							Tr		b,c
isobutyl tiglate	1094	1088					Tr				b,c
trans-sabinene hydrate	1095	1098					0.2				b,c
linalool	1100	1095	0.2		15.6	3.9	10.8	1.3		0.2	a,b,c
cis-thujone	1102	1101						0.1			a,b,c
n-nonanal	1105	1100								Tr	b,c
2-methyl butyl-2-methyl butyrate	1105	1100					0.2				b,c
hotrienol	1105	1103					0.1				b,c
isopentyl isovalerate	1108	1102					Tr				b,c
cis-rose oxide	1109	1106						3.3			b,c
endo-fenchol	1109	1114								Tr	b,c
2-methyl butyl isovalerate	1110	1103					0.2				b,c
phenyl ethyl alcohol	1112	1107							0.7		b,c
butanoic acid, 3-methyl-, 3-methyl-3-butenyl ester	1115	1115					Tr				b,c
trans-pinene hydrate	1116	1119								Tr	b,c

cis-p-menth-2-en-1-ol	1117	1118					0.2				b,c
trans-p-mentha-2,8-dien-1-ol	1117	1119								0.1	b,c
α-campholenal	1123	1122					Tr				b,c
trans-rose oxide	1125	1122						1.1			b,c
3-octanol acetate	1127	1120		0.1							b,c
limona ketone	1130	1137					0.4				b,c
cis-limonene oxide	1130	1132								Tr	b,c
cis-p-mentha-2,8-dien-1-ol	1132	1133								Tr	b,c
trans-pinocarveol	1132	1135					1.1				a,b,c
trans-limonene oxide	1135	1137								Tr	b,c
trans-p-menth-2-en-1-ol	1136	1136					0.2				b,c
camphor	1139	1141	0.1				0.1	0.2		Tr	a,b,c
neo-isopulegol	1141	1144	1.8					0.1			b,c
(3*Z*)-hexenyl isobutanoate	1146	1142								Tr	b,c
menthone	1150	1148	20.7	0.6	0.2			1.0			b,c
isoamyl tiglate	1150	1148				0.1	Tr				b,c
3-methyl-2-butenyl 3-methyl-butanoate	1151	1147					Tr				b,c
nerol oxide	1154	1154				0.1					b,c
citronellal	1154	1148						0.1		Tr	a,b,c
pinocarvone	1156	1160					1.0				b,c
borneol	1160	1165					0.3			0.3	a,b,c
iso-menthone	1161	1158	11.6	0.2	0.1			16.2			b,c
neo-menthol	1163	1161	5.3								b,c
δ-terpineol	1163	1162		0.1			0.1				b,c
hydrocinnamaldehyde	1164	1163							0.4		b,c
cis-pinocamphone	1168	1172					0.1				b,c
menthol	1169	1167	26.0	2.1	1.2						a,b,c
terpinen-4-ol	1172	1174	0.3	1			6.0				a,b,c
iso-menthol	1180	1179	0.7					0.1			b,c
p-cymen-8-ol	1183	1179					Tr				b,c
trans-p-mentha-1(7),8-dien-2-ol	1185	1187								Tr	b,c
neo-iso-menthol	1186	1184	0.1								b,c
α-terpineol	1187	1186	0.6	0.3	0.1		1.7	0.2		Tr	a,b,c

myrtenal	1191	1195					0.2				a,b,c
myrtenol	1191	1194					0.4				a,b,c
neo-dihydro carveol	1192	1193		0.1							b,c
cis-dihydro carvone	1193	1191		1.7						0.2	b,c
trans-dihydro carvone	1199	1200		0.2						0.9	b,c
methyl chavicol	1201	1195		0.1	77.9						b,c
trans-piperitol	1204	1207					0.1				b,c
octanol acetate	1215	1211	0.1								b,c
(Z)-cinnamaldehyde	1218	1219							0.4		b,c
iso-dihydro carveol	1218	1212								0.2	b,c
trans-carveol	1224	1215		0.3						0.3	b,c
nerol	1228	1227				2.5					a,b,c
hydrocinnamyl alcohol	1232	1227							0.1		b,c
citronellol	1233	1223						30.1			a,b,c
neoiso-dihydro carveol	1234	1226								0.5	b,c
pulegone	1235	1233	1.7								b,c
cis-carveol	1236	1226		0.2							b,c
cis-3-hexenyl-isovalerate	1238	1238	1.0								b,c
neral	1240	1235			0.2	Tr		0.2			a,b,c
carvone	1240	1239		58.2			Tr			35.2	a,b,c
o-anisaldehyde	1243	1242							0.8		b,c
hexyl isovalerate	1244	1243	0.1				Tr				b,c
cis-myrtanol	1247	1250					Tr				b,c
piperitone	1254	1249	1.4	0.4				0.2		1.1	b,c
cis-myrtanol	1255	1250						0.1			b,c
cis-piperitone epoxide	1255	1250								0.3	b,c
geraniol	1258	1249						1.9		0.1	a,b,c
cis-chrysanthenyl acetate	1259	1261					0.3				b,c
2-phenyl ethyl acetate	1260	1258							Tr		b,c
cis-carvone oxide	1264	1259								0.1	b,c
trans-ascaridol glycol	1265	1266					Tr				b,c
perilla aldehyde	1269	1269					Tr				b,c
isopiperitenone	1270	1271								tr	b,c
geranial	1272	1264			0.4			0.2		0.1	a,b,c
neo-menthyl acetate	1274	1271	0.1								b,c
n-decanol	1275	1266	0.2								b,c

(*E*)-cinnamaldehyde	1275	1270						82.7		a,b,c
trans-carvone oxide	1276	1273		0.1					0.1	b,c
citronellyl formate	1277	1271					9.1			b,c
neryl formate	1281	1280			0.1					b,c
bornyl acetate	1282	1287				0.6				a,b,c
hexyl-angelate	1288	1275			0.1					b,c
thymol	1289	1289				Tr	0.1		0.2	a,b,c
menthyl acetate	1293	1294	9.5	0.2						b,c
trans-pinocarvyl acetate	1296	1298				0.5				b,c
n-tridecane	1300	1300			Tr					a,b,c
carvacrol	1302	1298				Tr			0.5	a,b,c
geranyl formate	1303	1298					0.7			b,c
iso-menthyl acetate	1304	1304	0.2							b,c
(*E*)-cinnamyl alcohol	1304	1304						Tr		b,c
neoiso-isopulegyl acetate	1309	1312	0.1							b,c
nonyl acetate	1314	1313	0.1							b,c
myrtenyl acetate	1322	1324				Tr				b,c
(3*Z*)-hexenyl tiglate	1326	1319							Tr	b,c
iso-dihydro carveol acetate	1327	1326		0.2						b,c
δ-elemene	1331	1335				Tr			Tr	b,c
trans-carvyl acetate	1337	1339				Tr				b,c
piperitenone	1337	1340							2.1	a,b,c
hydrocinnamic acid	1341	1347						Tr		b,c
α-cubebene	1345	1345					0.1		Tr	a,b,c
citronellyl acetate	1355	1350					0.3			a,b,c
eugenol	1355	1356				0.2		Tr	Tr	a,b,c
cyclosativene	1360	1369				0.1				b,c
cis-carvyl acetate	1361	1365				0.2				b,c
piperitenone oxide	1362	1366		0.5						b,c
α-ylangene	1364	1373			0.4					b,c
neryl acetate	1365	1359			45.4					a,b,c
α-copaene	1368	1374				0.3	0.5	0.2	0.1	a,b,c
β-bourbonene	1377	1387	0.3	1.7		Tr	1.0		1.8	b,c
trans-myrtanol acetate	1380	1385				0.1				b,c
β-cubebene	1384	1387							0.2	b,c

geranyl acetate	1386	1379				0.1					b,c
β-elemene	1386	1389	0.1	0.1			0.1			1.0	a,b,c
iso-italicene	1390	1401				0.1					b,c
benzyl isovalerate	1392	1395					0.1				b,c
phenyl ethyl isobutanoate	1393	1393						0.1			b,c
italicene	1395	1405				3.4					b,c
(Z)-jasmone	1395	1392		0.1							b,c
n-tetradecane	1400	1400				Tr				tr	a,b,c
α-*cis*-bergamotene	1410	1411				2.5					b,c
(*E*)-caryophyllene	1410	1417	1.5	1.1	0.3		2.1	1.3		0.3	a,b,c
β-ylangene	1410	1420								0.3	b,c
decyl acetate	1413	1407	0.2								b,c
β-copaene	1420	1430		0.1			0.1			0.3	b,c
coumarin	1429	1432							0.7		a,b,c
α-*trans*-bergamotene	1431	1432			0.7	1.0	Tr			tr	b,c
α-guaiene	1432	1437						0.4			b,c
β-gurjunene	1435	1431								0.2	b,c
aromadendrene	1435	1439					Tr				a,b,c
6,9-guaiadiene	1437	1442				0.3		5.7			b,c
(*E*)-cinnamic acid	1437	1435							0.42		b,c
4,6,9-trimethyl-dec-8-e-3,5-dione	1439					0.8					b
(*Z*)-β-farnesene	1440	1440					0.2				b,c
cis-muurola-3,5-diene	1441	1448						0.4			b,c
octyl isovalerate	1441	1440	0.1								b,c
α-humulene	1443	1452		0.1	0.1	0.1	0.2			0.1	a,b,c
citronellyl propanoate	1445	1444						1.1			b,c
(*E*)-cinnamyl acetate	1445	1446							0.3		b,c
allo-aromadendrene	1451	1458					0.6	0.3		0.5	b,c
neryl propanoate	1456	1452				5.0					b,c
cis-cadina-1(6),4-diene	1456	1461					0.8	0.3		0.2	b,c
α-acoradiene	1458	1464				0.3					b,c
(*E*)-β-farnesene	1459	1454		0.5	0.1		0.4			0.6	a,b,c
dehydro-sesquicineole	1466	1469					Tr				b,c
β-acoradiene	1468	1469					0.1				b,c
α-amorphene	1473	1483				0.6					b,c

γ-curcumene	1476	1481				9.0	0.3				b,c
germacrene D	1476	1484	1.1	0.4			11.8	1.8	0.1	14.8	b,c
geranyl propanoate	1476	1476						0.5			b,c
ar-curcumene	1481	1479				7.6	0.7	0.1	Tr		b,c
trans-muurola-4(14),5-diene	1483	1493					Tr			Tr	b,c
phenyl ethyl 3-methyl butanoate	1484							0.1			b
δ-selinene	1486	1492				1.4					b,c
viridiflorene	1486	1496						1.0			b,c
bicyclogermacrene	1488	1500	0.3				0.7			0.6	b,c
α-zingiberene	1492	1493				0.1	0.3				b,c
α-muurolene	1494	1500				Tr	0.3	0.3	Tr	0.2	b,c
isodaucene	1495	1500								0.3	b,c
δ-amorphene	1500	1511				0.1	Tr				b,c
n-pentadecane	1500	1500								Tr	a,b,c
β-bisabolene	1506	1505				0.3			Tr	0.1	b,c
(*E*,*E*)-α-farnesene	1508	1505					1.9				a,b,c
cubebol	1507	1514								0.4	b,c
β-curcumene	1508	1514				0.3					b,c
geranyl isobutanoate	1515	1514						0.1			b,c
trans-calamenene	1517	1521				0.1			Tr		b,c
δ-cadinene	1517	1522	0.1			0.1	1.6	1.1	Tr	0.1	b,c
β-sesquiphellandrene	1519	1521					0.4				b,c
trans-cadina-1,4-diene	1524	1533						0.1			b,c
italicene ether	1525	1536				0.2					b,c
(*E*)-γ-bisabolene	1527	1529				0.1					b,c
citronellyl butanoate	1529	1530						0.8			b,c
(*E*)-*o*-methoxy cinnamaldehyde	1530	1529							10.1		b,c
α-calacorene	1535	1544				0.1	0.2				b,c
furopelargone A	1537	1538						0.1			b,c
(*E*)-α-bisabolene	1540	1540			1.4	0.1	0.1			0.1	b,c
germacrene B	1546	1559								Tr	b,c
β-calacorene	1555	1564					Tr				b,c
palustrol	1556	1567					0.4				b,c
geranyl butanoate	1562	1562						0.8			b,c

(*E*)-*p*-methoxy-cinnamaldehyde	1562	1562			0.3						b,c
(*E*)-nerolidol	1564	1563				Tr	Tr		Tr		a,b,c
spathulenol	1566	1577					0.2	0.1	0.1	0.4	b,c
caryophyllene oxide	1571	1582		0.1	0.1	0.3	0.9	0.3	Tr	0.1	a,b,c
ar-turmerol	1577	1582					0.2				b,c
globulol	1581	1590		0.1							b,c
2-phenyl ethyl tiglate	1582	1584						1.9			b,c
viridiflorol	1583	1592					12.6				a,b,c
salvial-4(14)-en-1-one	1583	1594								Tr	b,c
neryl isovalerate	1585	1582				0.8					b,c
guaiol	1591	1600				0.8					b,c
allo-cedrol	1592	1589					0.7				b,c
globulol	1592	1590					0.7				b,c
copaborneol	1593	1592					0.5				b,c
rosifoliol	1597	1600				3.7					b,c
geranyl isovalerate	1603	1606						0.1			b,c
1,10-*di*-*epi*-cubenol	1606	1618						0.1			b,c
10-*epi*-γ-eudesmol	1608	1622				0.1	1.0	1.3			b,c
eremoligenol	1619	1629				Tr					b,c
1-*epi*-cubenol	1619	1627					0.9	0.1			b,c
muurola-4,10(14)-dien-1-β-ol	1620	1630					0.9				b,c
cubenol	1623	1645				0.3					b,c
(3*Z*)-hexenylphenyl acetate	1630	1632	0.1								b,c
epi-α-muurolol	1633	1640					0.5			Tr	b,c
epi-α-cadinol	1634	1638						0.3			b,c
β-eudesmol	1639	1649				0.3		0.1			b,c
α-muurolol	1639	1644					0.1			Tr	b,c
α-eudesmol	1643	1652				0.3		0.1			b,c
α-cadinol	1646	1652					0.5	0.2		0.1	b,c
cis-calamenen-10-ol	1652	1660					0.4				b,c
trans-calamenen-10-ol	1660	1668					0.8				b,c
bulnesol	1559	1670				0.2					b,c
epi-β-eudesmol	1565					0.1					b
(*E*)-citronellyl tiglate	1666	1666						0.6			b,c

cadalene	1669	1675							Tr		b,c
eudesma-4(15),7-dien-1β-ol	1676	1687								0.3	b,c
epi-α-bisabolol	1678	1683				Tr	0.7				b,c
α-bisabolol	1680	1685					0.1				a,b,c
n-heptadecane	1699	1700								Tr	a,b,c
geranyl tiglate	1701	1696				0.1		0.6			b,c
n-pentadecanal	1713	1715								Tr	b,c
chamazulene	1716	1715					0.3				b,c
(6*R*,7*R*)-bisabolone	1741	1740					0.2				b,c
geraniol hexanoate	1754	1755								0.1	b,c
benzyl benzoate	1758	1759					Tr		0.1		b,c
hexahydrofarnesyl acetone	1844	1845					Tr			Tr	b,c
phenethyl-benzoate	1846	1844							0.1		b,c
n-octadecanol	2064	2070					Tr				b,c
13-hexyloxacyclotridec-10-en-2-one	2055								0.6		b
phytol	2118	2116					0.1				a,b,c
phenethyl cinnamate	2170	2158							Tr		b,c
n-tricosane	2300	2300					Tr				a,b,c
n-pentacosane	2500	2500					Tr				a,b,c
n-heptacosane	2700	2700					Tr				a,b,c
n-nonacosane	2900	2900					Tr				a,b,c
Total identified (%)			99.8	99.4	99.8	95.2	88.5	98.5	99.1	99.2	
Grouped compounds (%)											
Monoterpene hydrocarbons			12.3	27.8	0.2	2.3	12.3	7.5	0.1	32.9	
Oxygenated monoterpenes			81.0	66.7	18.3	57.8	29.1	71.5	Tr	42.8	
Sesquiterpene hydrocarbons			3.5	3.9	2.7	27.8	23.7	14.6	0.4	21.9	
Oxygenated sesquiterpenes				0.1	0.1	6.2	22.3	2.6	0.1	1.3	
Aromatics			0.1	0.1	78.3	0.2	0.2	2.1	97.8		
Others			2.9	0.7	0.1	0.9	0.3	0.2	0.6	0.3	

[a] Compounds are listed in order of their elution from a HP-5MS column.
[b] Linear retention index on HP-5MS column, experimentally determined using homologous series of C_8-C_{30} alkanes.

[c] Linear retention index taken from Adams (2007) or NIST 08 (2008) and literature.
[d] Relative percentage values are means of three determinations with a (RSD%) in all cases below 20%.
[e] Identification methods: a, based on comparison of RT, RI and MS with those of authentic compounds; b, based on comparison of mass spectrum with those reported in WILEY, ADAMS, FFNSC2 and NIST 08 MS libraries; c, based on comparison of calculated RI with those reported in ADAMS, FFNSC 2 and NIST 08.
[f] Tr, traces (% < 0.1).

Table 2. Acute toxicity of selected essential oils against 4th instar larvae of *Culex quinquefasciatus*.

Essential oil	LC_{50}	CI_{95}	LC_{90}	CI_{95}	Chi square
Achillea ligustica	89.5	85.2-95.1	120.2	109.9-140.2	0.301 n.s.
Lippia alba	59.6	54.3-65.5	107.7	94.2-129.9	3.355 n.s.
Cinnamomum verum	40.7	37.9-43.8	64.1	57.7-74.2	0.575 n.s.
Pelargonium odoratissimum	133.3	126.9-140.1	188.1	174.1-211.5	0.879 n.s.
Helichrysum italicum	234.1	199.1-356.1	570.1	485.6-598.8	0.289 n.s.
Ocimum basilicum	68.6	66.1-70.9	85.4	81.5-91.7	1.852 n.s.
Mentha spicata	88.2	83.2-93.8	127.6	116.8-144.5	4.492 n.s.
Mentha x *piperita*	218.7	120.9-136.5	200.8	184.9-224.1	1.496 n.s.

Concentration LC_{50} (LC_{90}) in µl L^{-1}causing 50% (90%) mortality of larvae for 24 h after application.
CI_{95} = 95% confidence intervals, essential oil activity is considered significantly different when the 95% CI fail to overlap.
Chi- square value: not significant (n.s., $P>0.05$).

Table 3. Acute toxicity of selected essential oils against *Musca domestica* adults.

Essential oil	LD_{50}	CI_{95}	LD_{90}	CI_{95}	Chi square
Achillea ligustica	121	118-126	219	215-232	1.251 n.s.
Lippia alba	115	110-119	183	170-192	1.253 n.s.
Cinnamomum verum	42	32-48	56	52-61	1.215 n.s.
Pelargonium odoratissimum	54	50-58	84	77-95	0.115 n.s.
Helichrysum italicum	42	33-49	84	75-91	0.744 n.s.
Ocimum basilicum	70	65-75	148	126-154	0.528 n.s.
Mentha spicata	86	82-89	107	102-119	0.315 n.s.
Mentha x *piperita*	59	51-64	143	121-184	0.506 n.s.

Doses LD_{50} (LD_{90}) in µg/adult causing 50% (90%) mortality of *Musca domestica* adults for 24 h after application.
CI_{95} = 95% confidence intervals, essential oil activity is considered significantly different when the 95% CI fail to overlap.
Chi- square value: not significant (n.s., $P>0.05$).

Table 4. Emergence and fertility of *Culex quinquefasciatus* adults that survived to a sub-lethal dose (LC_{30}=25 mg/l) of *Cinnamomum verum* essential oil administered to 4th instar larvae.

	Larval mortality (%)			Emergence (%)			Fecundity and fertility of adults		Larvae/all survived female (n)
	At 24 h	At 48 h	Total	Female	Male	Total adults	Average egg/female (n)	Emergence of eggs (%)	
Essential oil	19.3±2.6*	38.7±3.2**	45.7±5.1**	23.8±2.7**	19.1±2.6**	42.9±2.5**	103.2±3.1**	79.2±3.8**	1,945.2±168.5**
Control	0.0±0.0	0.8±0.1	12.8±3.2	41.3±2.3	38.8±4.9	80.1±7.3	126.9±3.5	97.3±0.8	5,097.3±128.1

Within each column, asterisks indicate significant differences among means ($P<0.05$).
n.s. = not significant.

Table 5. Longevity and fertility of *Musca domestica* flies that survived after adult exposure to a sub-lethal dose (LD_{20}=10 µg/adult) of *Cinnamonum verum* essential oil.

	Longevity (days)			Fecundity and fertility indicators		Natality	
	Female LT_{50} (CI_{95})	Male LT_{50}(CI_{95})	Chi square	Eggs/female (n)	Egg hatchability (%)	Adults from 100 eggs (n)	Inhibition of natality (%)
Essential oil	16.2 (15.9-17.3)	12.7 (11.5-13.2)	2.354 n.s.	261.1±28.3**	73.9±5.5**	52.2±5.3**	41.1±2.3
Control	18.5 (17.9-19.3)	13.8 (12.7-15.9)	3.231 n.s.	351.7±32.7	92.5±2.9	88.5±1.9	-

Within each column, asterisks indicate significant differences among means (P<0.05).
n.s. = not significant.

Figure

Fig. 1. The eight plant species from which the insecticidal essential oils were obtained.

Fig. 2. GC-MS chromatograms of the essential oils from *Cinnamomum verum* (a), *Lippia alba* (b), *Mentha spicata* (c), and *Ocimum basilicum* (d).

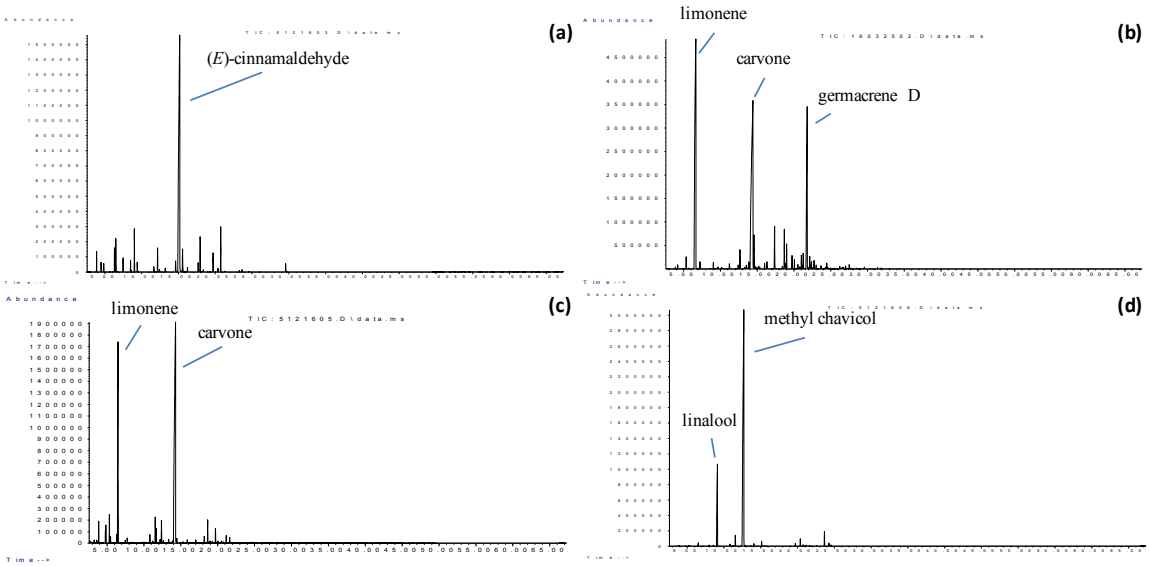

Fig. 3. Structures of the two major constituents in the essential oils from *Mentha* x *piperita* (a), *Mentha spicata* (b), *Ocimum basilicum* (c), *Helichrysum italicum* (d), *Achillea ligustica* (e), *Pelargonium odoratissimum* (f), *Cinnamomum verum* (g), and *Lippia alba* (h).