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Marek Chajduk, Marek Gołębiowski, Marzena Stańska, Christophe Lucas. Repellent effect of selected essential oils against the wolf spider *Pardosa hortensis* (Araneae: Lycosidae). *Behavioural Processes*, 2025, 233, pp.105283. <10.1016/j.beproc.2025.105283>. <hal-05360905>

HAL Id: hal-05360905

<https://hal.science/hal-05360905v1>

Submitted on 12 Nov 2025

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HAL Authorization

1 **Title:** Repellent effect of selected essential oils against the wolf spider *Pardosa hortensis*
2 (Araneae: Lycosidae)

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26 **Abstract**

27 Spiders play a vital role in ecosystem balance, contributing to insect population control and
28 biodiversity maintenance. However, their presence can cause fear and anxiety in humans,
29 particularly those affected with arachnophobia. As spiders increasingly appear in human
30 environments due to global climatic changes, even non synanthropic species, there is a growing
31 need for effective and natural repellents - not only to reduce human discomfort but also to
32 protect spiders by encouraging them to avoid urban areas and reducing unnecessary fatalities.
33 In this study, we investigated the potential repellent properties of ten essential oils against
34 female wolf spiders *Pardosa hortensis* (Araneae: Lycosidae), a very common grass species
35 from the second-largest spider genus in the world that may occasionally venture into human
36 surroundings. Through chemical analysis and binary choice behavioral tests, we identified five
37 oils - catnip, cedarwood, cinnamon, citronella, and clove - that significantly deterred spider
38 activity. Chemical constituents such as eugenol, β -caryophyllene, and geraniol likely contribute
39 to these repellent effects. While further research is needed to validate these findings across
40 diverse spider species, our study highlights the potential of essential oils and essential oil
41 yielding plants as natural spider repellents, either at the entrances of houses or as natural garden
42 defenses with specific plants. Altogether, it gives promise for arachnid management strategies
43 and for mitigating conflicts between humans and spiders.

44 **Keywords** essential oil, Lycosidae, deterrence, pest management, binary choice test,
45 arachnophobia

46 **Highlights**

- 47 • Five essential oils showed repellency effect against *Pardosa hortensis* spiders
- 48 • GC-MS analysis revealed a total of 113 compounds in ten essential oils
- 49 • Repellency was not affected by any variable, e.g. temperature or presence of cocoon
- 50 • Results support plant-based strategies for spider population control

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56 1. Introduction

57 Spiders play a crucial role within various ecosystems, acting as highly effective predators that
58 contribute significantly to the regulation of insect population and maintenance of biodiversity
59 (Marc et al. 1999; Guo et al. 2018). Their positive impact on both environmental sustainability
60 and human well-being is undeniable. The feeding habits of spiders, help to prevent the
61 outbreaks of pests contributing to population stabilization within agroecosystems (Riechert and
62 Lockley 1984; Dong et al. 2024). Through the strategic integration of spiders in biocontrol
63 initiatives, a significant reduction in pesticide usage has been achieved, resulting in successful
64 pest management strategies (Maloney et al. 2003). Furthermore, in certain environments,
65 spiders help mitigate disease spread by controlling the populations of mosquitoes and other
66 insect vectors (Lawania et al. 2013).

67 The matter of environmental impact of spiders is not as straightforward as it initially appears.
68 It is essential to acknowledge that, despite the overall positive impact of spiders, certain species
69 may exhibit a top-down effect on plants, decreasing seed production due to predation on
70 pollinators (Suttle 2003). Additionally, an important consideration in this context involves the
71 impact of interactions with spiders on the mental and emotional states of humans. Although
72 only a minimal percentage (0.5% or less) of all spider species may pose potential risks to
73 humans (Hauke and Herzig 2017), spiders are ranked among the top five most feared animals
74 (Davey 2008). The intense and uncontrollable fear of spiders, known as arachnophobia, is a
75 common phobia that affects a global population ranging from 2.7% to 6.1%, mainly among
76 women (Landová et al. 2021; Oosterink et al. 2009). Furthermore, the intensity of
77 arachnophobia varies among countries, reaching up to 9.5% in Hungary (Polák et al. 2020)
78 while feelings of anxiety, nervousness, or varying degrees of disgust and fear in the presence
79 of spiders, impact approximately a quarter of the UK population (Davey 2008; Gerdes et al.
80 2009).

81 Thin-legged wolf spiders, belonging to the *Pardosa* genus, can be found in a variety of
82 environments, such as grasslands, waste areas, and woodland clearings (Hanggi et al. 1995,
83 British Arachnological Society 2024). *Pardosa* species such as *Pardosa hortensis* (Araneae:
84 Lycosidae), which generally inhabit natural and agricultural environments, are not typically
85 found in areas dominated by humans. However, they may occasionally enter gardens and urban
86 settings, leading to fear in individuals with arachnophobia (Hanggi et al. 1995, Rozwałka 2006).
87 This applies in particular to global climatic changes where biological invasions are expected to
88 increase in urban areas (Bellard et al. 2017). Therefore, it is important to understand the factors

89 that affect spider behavior, particularly as the *Pardosa* genus is the second most diverse spider
90 genus, containing over 550 species that account for more than 1.5% of all documented spider
91 species. They also play a key role in agricultural systems (World Spider Catalog 2024). Further
92 knowledge about their exploration behavior and how to control it could help decrease potential
93 future conflicts with humans.

94 Contact with spiders may present adverse consequences, extending beyond the psychological
95 stress caused by the presence of a spider, particularly in individuals affected with
96 arachnophobia. Furthermore, spiders, which carry pathogenic and often antibiotic-resistant
97 bacteria on their body surfaces and chelicerae, can potentially act as vectors for infections and
98 diseases (Dunbar et al. 2020). These considerations, combined with the presence of venomous,
99 synanthropic species, such as black widow (*Latrodectus mactans*), whose bites may lead to
100 serious consequences, including death, underscores the importance of developing effective and
101 natural spider repellents. Implementing such repellents could help minimize both the
102 physiological and psychological risks related with human-spider interactions.

103 Essential oils (EO) are blends of volatile compounds extensively investigated for their repellent
104 properties, particularly against various insects and ticks, with notable success observed for a
105 variety of oils, including eucalyptus, red thyme and oregano EO (Adak et al. 2020; Costa et al.
106 2022a; Gaire et al. 2017a; Soutar et al. 2019). Despite this, the field of spider repellents has not
107 received comparable scientific attention, even though there is an evidence suggesting that
108 specific essential oils possess deterrent effects on spiders (Fischer et al. 2018). Essential oils
109 can act as spider repellents through two main mechanisms: the detection of volatile compounds
110 and contact toxicity. Volatile compounds released by certain plants, such as peppermint and
111 chestnut, cause spiders to quickly avoid areas where they have been applied (Fischer et al.
112 2018). Some oils (e.g. wintergreen or peppermint) can also cause physiological stress or death
113 to spiders when they come into direct contact with them, possibly by disrupting cuticle or
114 respiratory function (Ewing et al. 2019). While the exact mechanisms remain unclear, the
115 observed behavioural and toxic effects suggest that essential oils interfere with spiders' sensory
116 perception.

117 This research was undertaken in response to a recognized knowledge gap, and its main objective
118 was to evaluate the repellent properties of 10 essential oils on female *P. hortensis*, allowing us
119 to better understand the role of essential oils and plants that produce them in reducing
120 interactions with arachnids. These natural repellents may provide arachnophobes with a way to
121 reduce spider encounters through self-application or strategic placement of plants, facilitating

122 garden design that minimizes interactions with spiders while supporting these beneficial
123 biological control agents.

124 **2. Material and methods**

125 *2.1. Biological material.*

126 Adult females of wild *P. hortensis* (n=331) were hand-collected between May and June 2023
127 on a forest pathway in the University Park of Tours (Parc de Grandmont, France
128 47.35116780357453, 0.7019360398122906) between 4:00-6:00 pm daily. The pathway's wood
129 chip surface (non-natural) was made from local wood. The species was identified using existing
130 identification keys (Locket et al. 1974, Nentwig et al. 2023). Due to the breeding season during
131 which the spiders were collected, some of them carried cocoons. This variable was taken into
132 account in further statistical analysis. Spiders were then separately transferred into 50 ml Falcon
133 tubes (30 mm diameter) with a damp piece of cotton wool to provide water, and were kept
134 overnight at a temperature of $20 \pm 2^\circ\text{C}$ under a natural light cycle. Behavioral testing took place
135 the following day between 10:30 am to 3:30 pm utilising spiders that had been randomly
136 selected.

137 *2.2. Essential oils.*

138 Commercially available essential oils were sourced from 10 distinct plants (Etja company). The
139 selection of oils was based on their potential repellent properties against spiders or insects,
140 relying on information from manufacturer leaflets, online forums, and scientific literature
141 (Fischer et al. 2018; Gloríková et al. 2022). The chosen essential oils were derived from catnip
142 (*Nepeta cataria*), cedarwood (*Cupressus funebris*), cinnamon (*Cinnamomum zeylanicum*),
143 citronella (*Cymbopogon winterianus*), clove (*Syzygium aromaticum*), eucalyptus (*Eucalyptus*
144 *globulus*), lavender (*Lavendula angustifolia*), lemon (*Citrus limonum*), peppermint (*Mentha*
145 *piperita*), tea tree (*Melaleuca alterinifolia*).

146 To examine the full composition, three dichloromethane solutions of each essential oil were
147 prepared with analyte concentration of 1 mg/ml. Samples were analyzed by GC-MS on a QP-
148 2010 SE instrument (Shimadzu) equipped with a fused silica capillary column (RTX-5, 30 m x
149 0.25 mm i.d., film thickness 0.25 μm). The column oven temperature was maintained at 50°C
150 for 3 min, then increased to 250°C at $4^\circ/\text{min}$ with helium serving as the carrier gas at a column
151 head pressure of 100 kPa, and split ratio of 1:25. The injection temperature was set at 250°C ,
152 the interface temperature at 250°C , and the ion source temperature at 200°C . Mass spectra were
153 acquired in full scan mode with a step size of 0.1 amu, a scan lapse of 300 ms, and a range of

154 45-650 amu. Three samples for each oil were prepared and 3µl of each sample was injected 3
155 times as technical replicates. Identifying essential oil constituents involved comparing the
156 Kovat's Index (KI) of the analyzed compounds with standards and referencing the characteristic
157 ions in the mass spectra. Additionally, compounds were identified based on their mass spectra,
158 which were interpreted via fragmentation analyses and/or compared with spectra obtained by
159 the National Institute of Standard and Technologies Library NIST MS Search 2.3 (NIST11 and
160 NIST11b) and according to previous published data.

161 2.3. Repellent bioassays.

162 The effect of essential oils on *P. hortensis* spiders was investigated through binary choice tests,
163 adapted from previously published methodology (Fouche et al. 2018) originally designed for
164 Diptera larvae (Fig. 1). For the experiment, 100 µl of each essential oil was dissolved in acetone
165 to create a 10 ml solution at 1% - a commonly used concentration in studies on essential oil
166 repellence (Costa et al. 2022; Gloricova et al. 2022; Wagan et al. 2016). This dissolution
167 enabled us to apply the essential oil over a larger surface area compared to using it in its pure
168 form to cover the tested arena. Following the preparation step, the solutions were anonymized.
169 A filter paper (Whatman®, Grade 1) with a total diameter of 11 cm was halved and placed
170 inside a glass Petri dish measuring 11 cm in diameter and 2 cm in height. One half of the filter
171 paper was impregnated with 0.72 ml of the 1% essential oil solution, distributed evenly across
172 9 equally-spaced spots with 80 µl each, resulting in an oil concentration of 0.15 µg/cm². The
173 other half was impregnated with 0.72 ml of the carrying solvent as a control (acetone). Then,
174 impregnated filter papers were left under a fume hood for an hour, to allow solvent evaporation.
175 The selection of the essential oil solution and the side impregnated with the oil was done
176 randomly under double-blind conditions (Gamboa et al., 1991).

177 Headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-mass
178 spectrometry (GC-MS) analyses were conducted to confirm complete acetone evaporation used
179 for essential oils dilution. In the setup, four filter papers were halved and placed in a Petri dish.
180 Each half was impregnated with 0.72 ml of acetone. The dishes were placed under the fume
181 hood for 1, 5, 30, and 60 minutes, respectively. The test was repeated three times. Then, filter
182 papers were transferred to 20 ml glass vials with PTFE/silicone septa for headspace sampling
183 using 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber
184 (Merck). Sorption lasted for 60 minutes at 21 °C. The same GC-MS system and GC column
185 used for EO analysis were employed. Desorption proceeded in the GC injector for 2 minutes at
186 250 °C, with helium flow maintained at a constant pressure of 100 kPa and split ratio of 1:10.

187 The temperature program was: 40 °C for 2 minutes, then ramped to 250 °C at 4 °C/min. The
188 transfer line temperature was 250 °C, and the MS operated in positive ionization mode at 70
189 eV and 200 °C. Mass spectra were acquired in full scan mode with a step size of 0.1 amu, a
190 scan lapse of 300 ms, and a range of 45-650 amu. Acetone was identified by its characteristic
191 ions.

192 Following this preparation, a naïve spider that had been randomly selected was introduced into
193 the Petri dish. The first 30 minutes were designated for spider acclimatization without
194 recording. Subsequently, over the next 30 minutes, the spider's movements were recorded, with
195 one frame captured every 30 seconds using video cameras (Basler acA1300 – 60gc) driven by
196 the software Labview v16.0. The experiments were conducted at a controlled temperature of 20
197 \pm 1.5°C, under continuous light, with a relative humidity of 60 \pm 2%. A total of 311 *P. hortensis*
198 females were tested, with each oil assessed on 28 to 33 spiders. Videos were recorded in random
199 order under double-blind conditions (Gamboa et al. 1991). Analyses were done using the video-
200 tracking Behavioral Observation Research Interactive Software (BORIS) v.8.20 (Friard &
201 Gamba 2016).

202 2.4.Data analysis.

203 The spiders were localized on each side for each movie composed of 60 pictures (one picture
204 every 30 s over 30 min). After analyses, the anonymity was removed to determine the nature of
205 each side (either control or oil). Ratios were calculated with the number of pictures where
206 spiders were on the solvent divided by the total number of pictures, called the Repulsion Index.
207 Therefore, a high value of the ratio indicates a higher repellent effect. Data was analyzed using
208 a generalized linear mixed model (GLMM with quasibinomial distribution) with the
209 explanatory variables oil type and presence or absence of cocoons. The normality of the
210 residuals was plotted and checked. Other factors like temperature and humidity during the
211 behavioral test, the day of the behavioral test (one day after the collection), and the side where
212 oils were deposited (left or right) were also noted and tested. Because no effect of these factors
213 was significant, they were removed from further analyses. The Repulsion Index of each oil was
214 compared to a reference distribution of 0.5 using the `cbind()` function in R and analyzed using
215 a generalized linear mixed model (GLMM with quasibinomial distribution) to assess whether
216 the Repulsion Index values significantly deviate from the expected distribution represented by
217 0.5. Statistics were conducted using packages `car`, `emmeans`, `lme4`, `multcompView`,
218 `predictmeans`, `DHARMA`, and `ggplot2`.

219 3. Results

220 3.1. Essential oil chemical analyses.

221 The chemical compositions of the essential oils (EO) are presented in Table 1 and Figure 2. The
222 quality and quantity of the compositions of all the essential oils were uniform across all three
223 injections. Therefore, the standard deviation (SD) values have been omitted in Table 1 to
224 enhance its clarity. Note that the highest SD value was observed in limonene within lemon
225 essential oil with a value of 0.11.

226 A total of 113 organic compounds were detected through GC-MS analysis across all 10 essential
227 oils. The identified compounds range from 95.7% in cedarwood EO to 99.8% in eucalyptus EO.
228 Tea tree EO exhibited the highest number of compounds, totaling 38, while lemon EO showed
229 the lowest count, with only 7 compounds. In general, non-oxidized and oxidized monoterpenes
230 emerged as primary constituents, constituting over 80% of nearly all essential oils. Exceptions
231 were present in cinnamon EO, where these compounds accounted for 51.1%, and cedarwood
232 EO, which displayed a composition dominated by non-oxidized sesquiterpenes (75.1%) and
233 oxidized sesquiterpenes (19.1%).

234 In the cases of clove, lemon, and eucalyptus essential oils, one constituent significantly
235 dominated, namely eugenol (82.2%), limonene (90.2%), and eucalyptol (86.4%), respectively.
236 However, in other oils, no compound constituted more than half of the total mixture. Instead,
237 two to four major components were present at relatively high concentrations (10-45%). These
238 main constituents include menthol, menthone, and isomentone in peppermint EO; 4-terpineol
239 and γ -terpinene in tea tree EO; citronellal and geraniol in citronella EO; cis-thujopsen, α -
240 cedrene, and cedrol in cedarwood EO; trans cinnamaldehyde and eugenol in cinnamon EO;
241 nerol, geraniol, geranial, β -caryophyllene and neral in catnip EO; and linalyl acetate and linalool
242 in lavender EO. Additionally, almost all essential oils (8 out of 10) contained traces of α -pinene,
243 camphene, and limonene, and the majority (from 6 to 7 out of 10) exhibited the presence of β -
244 pinene, β -myrcene, o-cymene, linalool, α -terpineol, eucalyptol, β -caryophyllene, and α -
245 humulene (Table 1).

246 3.2. Repellent bioassays.

247 The repellent effects of essential oils (EO) on *P. hortensis* female spiders are summarized in
248 Table 2, and illustrated in Figure 3. The presence or absence of cocoons carried by spiders had
249 no significant effect on their behavior ($p=0.019$) and no interactions with EO were noticed ($p=$
250 0.092). The data demonstrate that five out of ten tested substances exhibit robust repellent

251 properties against spiders (no EO showed a significant attractant effect). Specifically, cinnamon
252 EO (n = 32, p < 0.0001), clove EO (n = 28, p < 0.0001), and catnip EO (n = 31, p < 0.0001)
253 demonstrate a very effective effect with a Repulsion Index of 0.99, with 1 representing the
254 maximum value. Additionally, significant repellence effects were observed for cedarwood EO
255 (n = 31, p = 0.001) and citronella EO (n = 29, p = 0.001), with corresponding Repulsion Index
256 values of 0.83 and 0.81, respectively. In contrast, statistical analysis revealed no significant
257 impact of the remaining five essential oils on spider behavior where spiders exhibit a relatively
258 even distribution between essential oils and control sides (lower p=0.178). Consequently,
259 peppermint, lavender, eucalyptus, tea tree, and lemon EO cannot be considered as repellents or
260 attractants for *P. hortensis* spiders.

261 HS-SPME-GC-MS analysis (Fig. 4) demonstrates that the amount of acetone on the filter paper
262 decreases with evaporation time. After 30 minutes, the acetone is already almost absent, and
263 after 60 minutes no acetone is detectable. Therefore, it can be concluded that acetone used as a
264 solvent in EO solutions is no longer present in the arena and, therefore, it could not influence
265 the behavior of the tested spiders.

266 **4. Discussion**

267 In this study, we investigated the repellent activity of these substances against spiders through
268 binary choice tests using 1% solutions on *P. hortensis* females. Our findings highlight that five
269 of these oils, namely catnip, cedarwood, cinnamon, citronella and clove significantly influenced
270 spider behavior. Consequently, these oils are potential candidates for natural spider
271 management. Moreover, the presence or absence of a cocoon carried by females did not impact
272 their behavioral response to these tests.

273 Scientific research on the repellent properties of essential oils against spiders has been relatively
274 limited, and only a few studies identifying specific oils and their components with such
275 potential (Calbiague et al. 2017; Fischer et al. 2018; Gloríková et al. 2022; Junker et al. 2011).
276 In a recent study by Glorikova et al. (2022), examining 15 essential oils on synanthropic
277 *Cheiracanthium mildei*, where clove EO exhibited the strongest repellent effect among all
278 substances tested. Earlier research has also reported the repellent properties of clove EO against
279 various insects (Hazarika and Khanikor 2022; Nerio et al. 2010; Yang 2009). The observed
280 increased repellency can be attributed to the substantial concentration of eugenol in clove EO,
281 constituting 82.2% of the extract in this study (Table 1). Eugenol's insecticidal activity involves
282 its primary targeting of octopamine receptors, leading to changes in heart-beat frequency and

283 cAMP production in the nervous system, reducing the walking ability of insects (Enan 2001).
284 It is important to note, however, that the efficacy of clove EO as a general arthropod repellent
285 cannot be universally confirmed. A study by Calbiague et al. (2017) investigated the poisonous
286 spider species Chilean recluse spider (*Loxosceles laeta*), and the results indicated that these
287 spiders did not exhibit a significant response to general odors, including eugenol. It is
288 noteworthy that in the context of essential oils, the secondary components can play a key role
289 in their overall activity, often exerting a synergistic effect with the action of the major
290 constituents (Dhifi et al. 2016).

291 The significant repellent effect observed here in *P. hortensis* spiders for cinnamon EO may also
292 be explained by the high concentration of eugenol (44.1%) present in the oil (Table 1). While
293 cinnamon EO has demonstrated potent repellent activity against diverse insect species (Marsin
294 et al. 2020; Prajapati et al. 2005) a different scenario was in the case of *C. mildei* spiders, where
295 this substance did not exhibit statistically significant repellent properties (Glorikova et al.
296 2022). However, in this case, the disparity may not necessarily be connected to taxonomic
297 differences but rather to the lower concentration of eugenol, accounting for only 1.33% of the
298 extract. Thus, the discouraging activity of essential oils varies not only within taxa (Fischer et
299 al. 2018) but also due to alterations in composition, either occurring naturally or resulting from
300 sourcing and production differences.

301 In a study by Junker et al. (2011), a binary choice experiment involving *Pisaura mirabilis* and
302 *Misumena vatia* spiders demonstrated that the repellent activity of β -caryophyllene is
303 contingent upon the taxa involved. The relatively high concentrations of this compound in
304 essential oils significantly affect *P. hortensis* spider behavior, specifically catnip EO (10.3%),
305 clove EO (8.4%), and cinnamon EO (4.1%), suggesting that β -caryophyllene may deter a
306 broader range of spider species. However, in the research on deterrent activity of *Mikania*
307 *micrantha* essential oil compounds on *Plutella xylostella* showed that β -caryophyllene was the
308 only compound that didn't have a significant effect on insects (Zhang et al. 2008). This
309 underscores the importance of testing its repellent properties on a wider range of arthropod
310 species.

311 Geraniol, found in relatively high concentrations in *P. hortensis* repellent essential oils from
312 catnip (17.8%) and citronella (27.1%) (Table 1), exhibit as a notable compound. Both essential
313 oils have been demonstrated to possess repellent properties against insects (Nerio et al. 2010;
314 Nguyen et al. 2018). Additionally, geraniol itself, along with geranyl acetate contributing 6.1%
315 of citronella EO (Table 1.), exhibits strong insecticidal activity, including ovicidal effects

316 against *Musca domestica* (Rice and Coats 1994). The strong correlation observed between
317 relatively high concentrations of these compounds and the significant deterrent properties of
318 catnip and citronella essential oils against *P. hortensis* females suggests their key role in spider
319 repellency (Nerio et al. 2010; Nguyen et al. 2018; Rice and Coats 1994).

320 None of the previously mentioned compounds were present in cedarwood EO, yet this
321 substance showed strong repellent properties against *P. hortensis* females. Although cedarwood
322 EO, like many others, has not been specifically tested for spider repellency, its effectiveness
323 against other arthropods has been confirmed. Cedarwood EO, with its main constituent, α -
324 thujopsen, which constitutes 31.6% of the oil (Table 1), demonstrated repellent effects on
325 *Coptotermes formosanus* termites (Zhu et al. 2001). Furthermore, α -cedrene, a main ingredient
326 found in the lemon balm (*Melissa officinalis*) EO, comprising 22.3% of cedarwood EO (Table
327 1), is suspected to be responsible for its repellent properties against the spider mite *Tetranychus*
328 *urticae* (Momen et al. 2014). Additionally, cedrol, a major component of the *Cupressus*
329 *sempervirens* EO, accounting for 17.6% of cedarwood EO (Table 1), exhibits potent insecticidal
330 properties and biochemical effects against the *Culex quinquefasciatus* mosquito (Almadiy and
331 Nenaah 2021).

332 It is well known that spiders can easily detect both substrate-borne and airborne chemicals.
333 Relying mainly on mechanoreception and chemoreception, spiders use sensory hairs and
334 specialized structures on their legs and pedipalps to perceive environmental cues (Foelix 1970,
335 Gaskett 2007, Fischer 2019, Schulz 2009, Chajduk et al. 2024). Numerous studies show that
336 certain volatile compounds can trigger behavioral responses without direct contact, as seen in
337 responses to specific airborne sex pheromones in *Xysticus ferox* (Aldrich and Barros 1995),
338 *Pardosa milvina* (Searcy et al. 1999), and *Agelenopsis aperta* (Papke et al. 2001). Similarly,
339 contact-based organic compounds, such as semiochemicals found on webs, cuticles, or
340 substrates, also play roles in spider communication and behavior, as observed in *Allagelena*
341 *difficilis* (Chen et al. 2018), *Latrodectus hasselti* (Jerhot et al. 2010), and *Latrodectus hesperus*
342 (Johnson et al. 2011). Considering these findings, our results further support the idea that *P.*
343 *hortensis*, like other spider species, can detect and respond to certain airborne and/or contact
344 organic compounds. Determining which concentrations and in which conditions spiders could
345 detect both types of chemicals will be of major interest for spider management.

346 The chemical compositions of the essential oils from catnip (*N. cataria*) (Ibrahim et al. 2022),
347 cedarwood (*C. funebris*) (Lyu et al. 2019), cinnamon (*C. zeylanicum*) (Alizadeh Behbahani et
348 al. 2020), citronella (*C. winterianus*) (Simic et al. 2008), clove (*S. aromaticum*) (Trajano et al.

2010), eucalyptus (*E. globulus*) (Almas et al. 2021), lavender (*L. angustifolia*) (Kozuharova et al. 2023), lemon (*C. limonum*) (Ashmawy et al. 2019), peppermint (*M. piperita*) (Taherpour et al. 2017) and tea tree (*M. alterinifolia*) (Sevik et al. 2021) revealed by GC-MS analyses coincide with existing literature. Among the 10 essential oils evaluated, eucalyptus, lavender, lemon, peppermint and tea tree did not exhibit significant repellent properties against *P. hortensis* females. However, considering the potential variability in the impact of substances on spider behavior across taxa or due to differences in composition, it is inconclusive whether these essential oils would universally function as effective spider repellents. For instance, peppermint EO, showed no repulsive effects on *P. hortensis* (current study), *C. mildei* (Gloríková et al. 2022), and *S. grossa* spiders (Fischer et al. 2018) but notably deterred the *A. diadematus* and *L. geometricus* species (Fischer et al. 2018). On the other hand, differences in repellency effects among species were less visible for other essential oils. Lemon EO failed to deter *P. hortensis* (current study), *L. geometricus*, *S. grossa*, and *A. diadematus* spiders (Fischer et al. 2018). Additionally, limonene, constituting 90.2% of the oil (Table 1), did not demonstrate significant repellent properties against *M. vatia*, *P. mirabilis* (Junker et al. 2011), and *L. laeta* spiders (Calbiague et al. 2017), nor did it, as the main component of sweet orange (*Citrus sinensis*) EO (28.4%), exhibit repellent effects against *C. mildei* spiders (Gloríková et al. 2022). Similarly, lavender EO also did not deter both *P. hortensis* (current study) and *C. mildei* spiders (Gloríková et al. 2022). The main ingredients of this essential oil are linalyl acetate (39.5%) and linalool (32.3%) (Table 1). The former is one of the major components of anise (*Pimpinella anisum*) EO (10.4%), while the latter is the primary component of coriander (*Coriandrum sativum*) EO (60.0%), marjoram (*Origanum majorana*) EO (21.1%), and camphor tree (*Cinnamomum camphora*) EO (17.8%), none of which exhibited significant repellent properties against *C. mildei* spiders (Gloríková et al. 2022). Furthermore, linalool did not deter *M. vatia* and *P. mirabilis* spiders (Junker et al., 2011). A similar pattern is observed with eucalyptus EO, 86.4% of which consisted of eucalyptol (Table 1). Neither the essential oil, nor the individual substance, exhibited a repellent effect on *P. hortensis* (current study) or *L. laeta* spiders (Calbiague et al. 2017), respectively. Essential oils are secondary plant metabolites that were originally selected as defenses and signaling compounds against herbivores and microbes (Fischer et al. 2018, Ewing et al. 2019). Spiders frequently forage and settle in habitats that are rich in these volatiles, so they are naturally exposed to them. Some compounds may repel spiders as by-products of their defensive role. Furthermore, their pest-repellent properties may cause spiders to avoid a given area due to the lack of food, prompting them to seek alternative locations. On the other hand, other essential oils could indirectly attract them if they signal the

383 presence of prey. The variation in oil composition among plant species and tissues also creates
384 different outcomes, which explains why the same essential oil can have repellent, neutral or
385 attractive properties, depending on the spider species and ecological setting (Fischer et al. 2018,
386 Gloríková et al. 2022, Lee 2018).

387 When assessing the repellent efficacy of essential oils, the time factor is crucial. Essential oils
388 consist mainly of terpenes and terpenoids, volatile substances that can quickly evaporate from
389 surfaces during experiments, potentially reducing their impact on arthropod behavior.
390 Examination of the compositions of essential oils presented in Table 1 reveals certain
391 correlations between the dominant types of compounds and repellent properties. Interestingly,
392 essential oils with no significant deterrent effects often contain higher concentrations of more
393 volatile monoterpenes, while those with significant repellent properties mainly contain less
394 volatile sesquiterpenes. Consequently, the one-hour evaporation time of solvent may adversely
395 affect the repellent properties of essential oils with higher concentrations of more volatile
396 compounds. However, this issue is complex because the effectiveness of oils is influenced by
397 various factors, including the concentration of the tested substance, the solvent used for dilution,
398 and the unique chemical composition influenced by factors such as geographical location,
399 environmental conditions, and extraction methods (Barra 2009; Luker et al. 2023). In studies
400 on the repellency of various essential oils against mosquitoes, using similar techniques but
401 different concentrations and complex solvents, the protective effect lasted from less than 20
402 minutes to over 2 hours for four essential oils (Amer and Mehlhorn 2006; Luker et al. 2023).
403 Additionally, a study by Amer et al. (2006) found that three different solvents (ethanol, vanillin,
404 and a complex solvent), in the same essential oils and concentrations, resulted in different
405 repellent durations. Moreover, a study by Luker et al. (2023) showed that peppermint essential
406 oil provided longer protection than cedarwood and citronella essential oils, contrary to the
407 results obtained in our study. These results are important for the development of effective
408 repellent strategies in plans to protect against potential invasive species.

409 In summary, our binary choice tests on *P. hortensis* females revealed five essential oils as good
410 candidates for spider management strategies: catnip (*Nepeta cataria*), cedarwood (*Cupressus*
411 *funnebris*), cinnamon (*Cinnamomum zeylanicum*), citronella (*Cymbopogon winterianus*), and
412 clove (*Syzygium aromaticum*) essential oils. Comprehensive GC-MS analyses and a review of
413 existing literature have identified several terpene compounds, including eugenol, β -
414 caryophyllene, geraniol, geranyl acetate, α -thujopsene, α -cedrene, and cedrol, that may
415 contribute to these repellent effects, although further research is needed to confirm their roles.

416 In fact, it is difficult for now to tell which compound is the most active or if it is a combination
417 or even a specific ratio of two or more compounds that elicit the strongest response. Although
418 our study is one of the first attempts to identify substances capable of repelling spiders, the
419 effectiveness of oils in deterring specific spider species remains dependent on various factors,
420 including species diversity and chemical compositions. Therefore, it is difficult to definitively
421 classify the essential oils identified in this study as universally effective repellents for spiders
422 despite their very effective impacts on spider behavior in this experiment. In practice, this study
423 offers a promising application for people suffering from arachnophobia when planning gardens.
424 Through the strategic use of plants that produce repellent essential oils, spaces can be created
425 to naturally discourage spider from being present while supporting ecosystem health. This
426 plant-based approach not only increases the psychological comfort for arachnophobes but also
427 reduces spider mortality caused by actions caused by fear. Because spiders play an important
428 role in ecosystems, promoting the harmonious coexistence of humans and spiders is both
429 beneficial and achievable. Further studies of this type should be conducted on potentially
430 dangerous synanthropic spider species to create natural home-use repellents.

431 **Acknowledgments**

432 We thank Marina Millet for her help in collecting spiders for the behavioral tests.

433 **Author contributions**

434 **Marek Chajduk**: Conceptualization, Methodology, Formal analysis, Investigation, Resources,
435 Writing – Original Draft, Visualization. **Marek Gołębiowski**: Methodology, Formal Analysis,
436 Writing – Review & Editing, Supervision, Funding acquisition. **Marzena Stańska**: Data
437 Curation, Writing – Review & Editing. **Christophe Lucas**: Conceptualization, Methodology,
438 Software, Formal Analysis, Data Curation, Writing – Review & Editing, Supervision.

439 **Funding sources**

440 Financial support was provided by the Polish Ministry of Research and Higher Education under
441 the grant 531-T010-D594-25.

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666

667 **Figure legends**

668 Fig. 1. Experimental setup for the binary choice test, outlining the preparation of essential oils
669 solutions, the handling of individuals, and the recording of behavior events.

670 Fig. 2. Chromatograms of the various essential oils, scaled to the highest peak (relative
671 intensity). The main peaks are highlighted in red text.

672 Fig. 3. The repellent effect of 1% essential oil solutions (10 essential oils) on *P. hortensis*
673 females (sample size ranging from 28 to 33 spiders, depending on the oil). A higher Repulsion
674 Index reflects greater deterrent activity against spiders. The box plot displays the median, upper
675 and lower quartiles, range, outliers (dots out of range), and means (crosses).

676 Fig. 4. Chromatograms from HS-SPME-GC-MS analysis of filter papers impregnated with
677 acetone and allowed to evaporate for 1, 5, 30, 60 minutes, respectively. The mass spectrum
678 shown corresponds to acetone.

679

GC-MS

essential oil (EO)



(n=10)

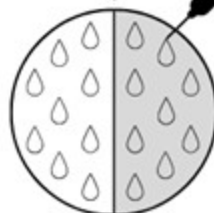
1% solution
in acetone

*Pardosa
hortensis*



♀ (n=28-33)

control



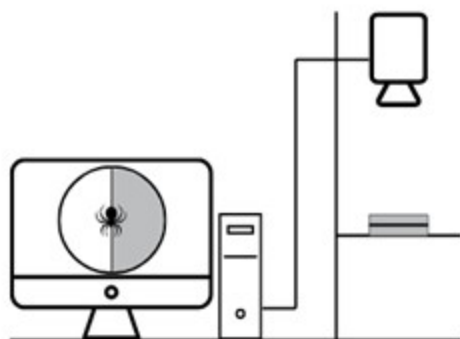
9 x 80 μ l

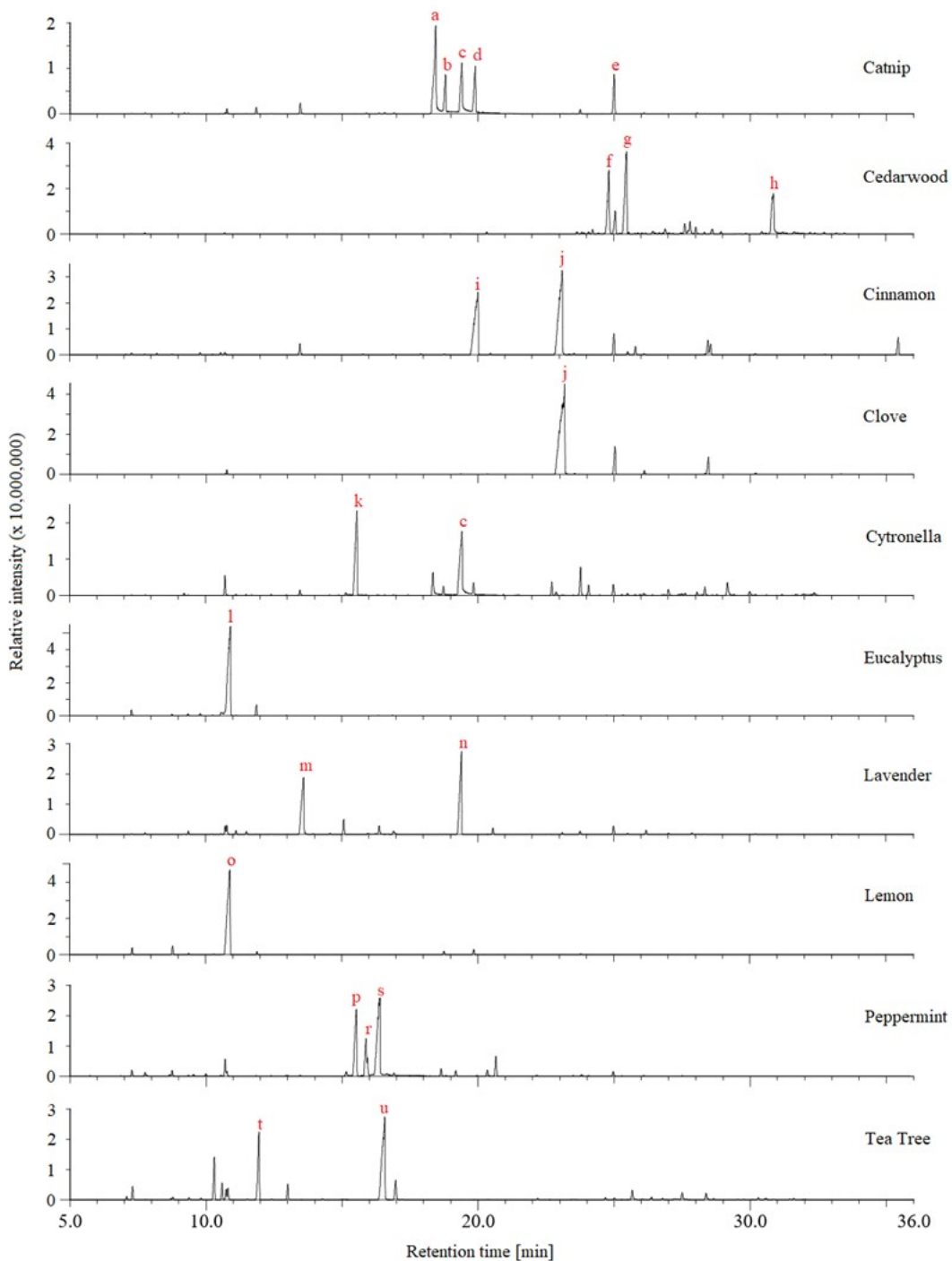
Petri dish \varnothing 11cm

24h

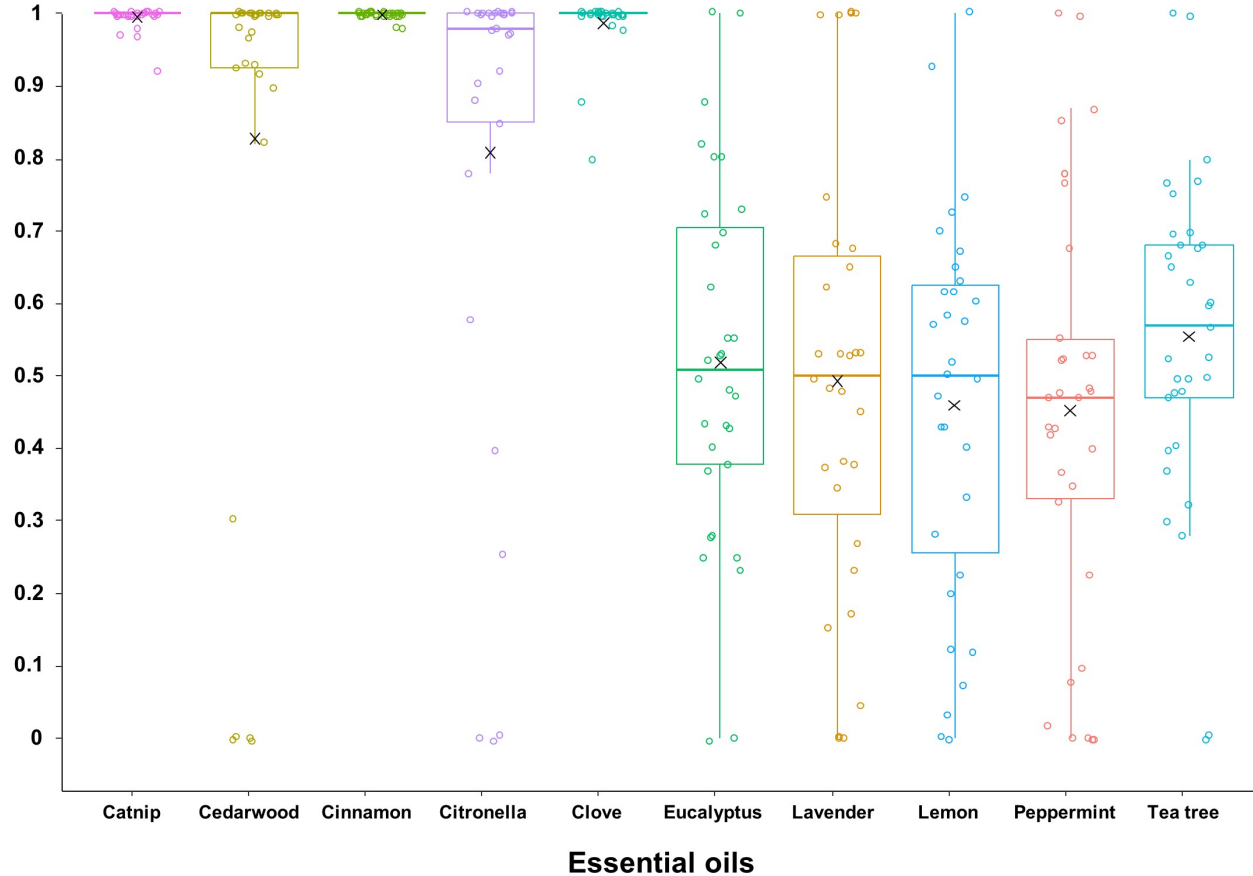
evaporation
1h

video recording



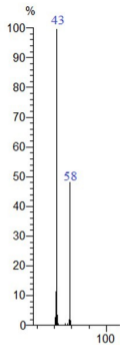


Repulsion Index



Relative intensity

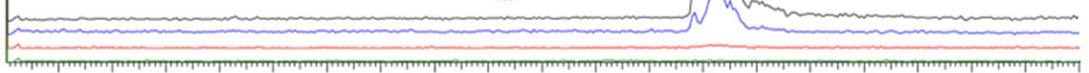
$2 \cdot 10^6$



0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2.0

Retention time (min)

1 min
5 min
30 min
60 min



680 Table 1. Chemical composition of 10 essential oils (in percentages with standard deviation in parentheses) analyzed via GC-MS: catnip (*N. cataria*),
681 cedarwood (*C. funebris*), cinnamon (*C. zeylanicum*), citronella (*C. winterianus*), clove (*S. aromaticum*), eucalyptus (*E. globulus*), lavender (*L.*
682 *angustifolia*), lemon (*C. limonum*), peppermint (*M. piperita*), and tea tree (*M. alterinifolia*). Concentrations exceeding 10% are highlighted in bold;
683 RT refers to retention time in minutes, and KI indicates the Kovats Index. The main peaks are labeled with letters corresponding to Figure 2. The
684 main peaks are labeled with letters corresponding to Figure 2.

RT	Compound	Main peak (Fig. 2)	KI	Catnip	Cedarwood	Cinnamon	Citronella	Clove	Eucalyptus	Lavender	Lemon	Peppermint	Tea tree
7.09	<i>α-Thujene</i>		930		0.13 (±0.02)								0.57 (±0.02)
7.33	<i>α-Pinene</i>		939	0.12 (±0.00)		0.29 (±0.02)		0.08 (±0.01)	2.32 (±0.05)	0.2 (±0.01)	2.46 (±0.05)	1.19 (±0.02)	2.44 (±0.07)
7.79	<i>Camphene</i>		954	0.18 (±0.02)	0.27 (±0.03)	0.09 (±0.01)	0.08 (±0.01)	0.06 (±0.01)		0.48 (±0.05)		0.67 (±0.03)	0.06 (±0.00)
8.21	<i>Benzaldehyde</i>		957			0.16 (±0.01)							
8.67	<i>Sabinen</i>		975									0.3 (±0.01)	0.19 (±0.01)
8.86	<i>β-Pinene</i>		979	0.12 (±0.01)		0.11 (±0.03)			0.57 (±0.02)	0.14 (±0.00)	2.59 (±0.06)	1.14 (±0.05)	0.46 (±0.02)
9.19	<i>3-Octanone</i>		982							0.17 (±0.03)			
9.38	<i>β-Myrcene</i>		990	0.12 (±0.03)			0.08 (±0.00)		0.7 (±0.03)	0.83 (±0.04)	0.46 (±0.04)	0.15 (±0.02)	0.39 (±0.03)
9.54	<i>3-Octanol</i>		991									0.32 (±0.03)	
9.80	<i>α-Phellandrene</i>		1002			0.32 (±0.04)			0.91 (±0.04)				0.26 (±0.03)
10.01	<i>δ-2-Carene</i>		1002									0.37 (±0.02)	
10.28	<i>α-Terpinene</i>		1017		0.13 (±0.01)	0.05 (±0.01)			0.28 (±0.03)				9.09 (±0.09)
10.59	<i>p-Cymol</i>		1024										3.03 (±0.07)
10.56	<i>o-Cymene</i>		1026	0.09 (±0.00)		0.32 (±0.03)		0.08 (±0.00)	3.01 (±0.05)	0.22 (±0.02)		0.16 (±0.02)	
10.75	<i>Limonene</i>	o	1029	0.25 (±0.03)	0.27 (±0.01)	0.34 (±0.04)	3.65 (±0.06)	0.10 (±0.01)		2.23 (±0.07)	90.17 (±0.11)	3.48 (±0.09)	
10.74	<i>Sylvestrene</i>		1030										2.01 (±0.07)
10.77	<i>Eucalyptol</i>	l	1031	0.96 (±0.04)				1.01 (±0.04)	86.43 (±0.09)	2.33 (±0.09)		0.85 (±0.03)	2.10 (±0.05)
11.11	<i>(Z)-β-Ocimene</i>		1037				0.15 (±0.03)		0.16 (±0.01)	1.01 (±0.04)			
11.52	<i>(E)-β-Ocimene</i>		1050	0.06 (±0.00)			0.13 (±0.02)		0.08 (±0.01)	0.76 (±0.01)			0.18 (±0.03)
11.88	<i>γ-Terpinene</i>	t	1059	1.31 (±0.06)					4.27 (±0.07)	0.11 (±0.00)			18.08 (±0.08)
12.42	<i>cis-Linalool oxide</i>		1072							0.12 (±0.01)			0.17 (±0.01)
12.99	<i>Terpinolene</i>		1088						0.26 (±0.03)	0.29 (±0.03)	0.89 (±0.03)	0.25 (±0.01)	
13.00	<i>Isoterpinolene</i>		1088										3.08 (±0.04)
13.47	<i>Linalool</i>	m	1096	2.62 (±0.05)	0.08 (±0.00)	2.02 (±0.02)	1.20 (±0.01)			32.26 (±0.07)		0.17 (±0.03)	0.06 (±0.00)
14.29	<i>(E)-p-Menth-2-en-1-ol</i>		1132										0.22 (±0.03)
14.77	<i>1-Terpinenol</i>		1133							0.37 (±0.01)			
15.08	<i>Camphor</i>		1141							4.28 (±0.06)			
15.16	<i>neo-Isopulegol</i>		1148				0.63 (±0.01)						
15.16	<i>Isopulegol</i>		1149										
15.53	<i>Menthone</i>	p	1152									1.15 (±0.02)	
15.56	<i>Citronellal</i>	k	1153				28.41 (±0.09)					24.47 (±0.08)	
15.88	<i>Isomenthone</i>	r	1162									10.27 (±0.07)	
15.89	<i>cis-Verbenol</i>		1164	0.19 (±0.03)									
15.94	<i>neo-Menthol</i>		1165									3.10 (±0.05)	

15.96	<i>Borneol</i>		1169						0.52 (±0.01)				
16.39	<i>Menthol</i>	s	1171										
16.39	<i>4-Terpineol</i>	u	1177									40.37 (±0.10)	
16.95	<i>α-Terpineol</i>		1188	0.22 (±0.01)	0.05 (±0.00)				0.16 (±0.01)	2.52 (±0.05)			40.12 (±0.10)
16.99	<i>Methyl salicylate</i>		1190					0.09 (±0.01)	0.31 (±0.02)	0.87 (±0.03)		0.55 (±0.04)	4.74 (±0.09)
17.18	<i>γ-Terpineol</i>		1199	0.25 (±0.01)									
17.91	<i>cis-Cinnamaldehyde</i>		1217		0.22 (±0.01)								
18.36	<i>Citronellol</i>		1225			5.29 (±0.05)							
18.44	<i>Nerol</i>	a	1227	38.01 (±0.10)									
18.64	<i>Pulegone</i>		1237									1.48 (±0.05)	
18.84	<i>Methyl thymyl ether</i>		1237		0.13 (±0.01)								
19.01	<i>Neral</i>	b	1238	10.31 (±0.06)		1.83 (±0.05)				0.93 (±0.01)			
19.18	<i>Piperitone</i>		1252									1.27 (±0.05)	
19.36	<i>Chavicol</i>		1247					0.23 (±0.01)					
19.40	<i>Linalyl acetate</i>	n	1250							39.47 (±0.10)			
19.42	<i>Geraniol</i>	c	1252	17.80 (±0.09)		27.05 (±0.08)							
20.00	<i>trans-Cinnamaldehyde</i>	i	1267		35.68 (±0.09)								
20.09	<i>Geranial</i>	d	1267	14.57 (±0.09)		2.38 (±0.05)							
20.01	<i>Citronellyl formate</i>		1273							1.90 (±0.05)			
20.38	<i>Bornyl acetate</i>		1285		0.48 (±0.01)			0.11 (±0.01)				1.19 (±0.04)	0.07 (±0.00)
20.47	<i>Safrole</i>		1289			0.23 (±0.01)							
20.56	<i>Lavandulol acetate</i>		1291							1.71 (±0.04)			
20.65	<i>Menthyl acetate</i>		1295									4.50 (±0.09)	
22.19	<i>δ-Elemene</i>		1338									0.16 (±0.02)	0.33 (±0.01)
22.72	<i>Citronellyl acetate</i>		1354			2.75 (±0.03)							
23.11	<i>Eugenol</i>	j	1360			44.07 (±0.10)	0.79 (±0.01)	82.17 (±0.10)					
23.11	<i>Neryl acetate</i>		1362							0.52 (±0.01)			
23.54	<i>α-Copaene</i>		1374			0.24 (±0.01)		0.21 (±0.01)				0.07 (±0.00)	0.11 (±0.01)
23.78	<i>Geranyl acetate</i>		1381	0.92 (±0.00)		6.11 (±0.05)				0.94 (±0.05)			
23.65	<i>2-epi-α-Funebrene</i>		1382		0.63 (±0.01)								
23.80	<i>β-Bourbonene</i>		1388									0.24 (±0.01)	
23.82	<i>α-Duprezianene</i>		1388		0.48 (±0.02)								
24.08	<i>β-Element</i>		1390		0.56 (±0.02)		2.15 (±0.03)						
24.23	<i>α-Chamipinene</i>		1396		1.03 (±0.03)								
24.68	<i>α-Gurjunene</i>		1409										0.44 (±0.01)
24.82	<i>α-Cedrene</i>	f	1411		22.25 (±0.10)								
25.01	<i>β-Caryophyllene</i>	e	1419	10.32 (±0.07)		4.13 (±0.06)	2.56 (±0.05)	8.35 (±0.07)		2.54 (±0.05)		1.07 (±0.07)	0.42 (±0.02)
25.05	<i>β-Cedrene</i>		1420		6.34 (±0.07)								
25.47	<i>cis-Thujopsene</i>	g	1431		31.59 (±0.10)				0.34 (±0.01)				
25.50	<i>trans-α-Bergamotene</i>		1434				0.46 (±0.01)						
25.52	<i>Coumarin</i>		1436			0.49 (±0.01)				0.35 (±0.01)			
25.55	<i>γ-Element</i>		1436		0.35 (±0.01)								
25.66	<i>Aromandendrene</i>		1439										2.33 (±0.05)
25.79	<i>α-Guaiene</i>		1440									0.23 (±0.01)	
25.80	<i>Cinnamyl acetate</i>		1447			1.57 (±0.04)							
26.02	<i>cis-Muurolo-3.5-diene</i>		1449										0.15 (±0.01)
26.13	<i>α-Humulene</i>		1454	0.24 (±0.01)		0.22 (±0.01)	0.43 (±0.01)	1.00 (±0.04)				0.16 (±0.01)	0.11 (±0.01)
26.19	<i>(E)-β-Farnesene</i>		1459		0.25 (±0.03)					1.28 (±0.04)			
26.37	<i>Alloaromadendrene</i>		1460		0.90 (±0.00)								0.65 (±0.03)

26.89	<i>β</i> -Chamigrene	1476	1.49 (±0.05)									
27.01	<i>D</i> -Germacrene	1481			1.39 (±0.05)			0.46 (±0.01)				
27.21	<i>α</i> -Selinene	1498								0.25 (±0.01)		
27.50	<i>trans</i> - <i>β</i> -Guaiene	1502								1.77 (±0.06)		
27.61	<i>β</i> -Himachalene	1502	2.72 (±0.05)									
27.63	<i>α</i> -Muurolene	1502			0.50 (±0.02)					0.21 (±0.01)		
27.73	<i>α</i> -Chamigrene	1503	0.51 (±0.02)									
27.81	Cuparene	1504	2.94 (±0.06)									
27.88	<i>β</i> -Bisabolene	1509						0.47 (±0.03)				
28.01	<i>α</i> -Alaskene	1512	1.63 (±0.04)									
28.06	<i>γ</i> -Cadinene	1513	0.24 (±0.02)					0.20 (±0.03)				
28.36	<i>β</i> -Sesquihellandrene	1523						0.21 (±0.04)				
28.37	<i>δ</i> -Cadinene	1523			1.85 (±0.05)	0.20 (±0.01)				1.48 (±0.06)		
28.46	Eugenol acetate	1525		2.70 (±0.06)		5.17 (±0.06)						
28.56	<i>o</i> -Methoxycinnamaldehyde	1526		2.08 (±0.06)								
28.62	<i>γ</i> -Cuprenene	1533	1.39 (±0.05)									
29.18	Elemol	1549			3.52 (±0.06)							
30.00	Germacrene- <i>D</i> -4- <i>ol</i>	1575			1.02 (±0.04)							
30.10	Spathulenol	1577								0.16 (±0.00)		
30.20	Caryophyllene oxide	1584		0.21 (±0.01)	0.19 (±0.02)	0.44 (±0.03)		0.23 (±0.02)				
30.30	Globulol	1590								0.50 (±0.03)		
30.56	Viridiflorol	1592	0.70 (±0.01)							0.26 (±0.03)		
30.59	Ledol	1602								0.18 (±0.02)		
30.87	Cedrol	1600	17.59 (±0.09)									
31.63	<i>α</i> -Acorenol	1635	0.46 (±0.02)									
31.96	<i>epi</i> - <i>α</i> -Cadinol	1639						0.14 (±0.02)				
32.02	Cubenol	1645			0.59 (±0.03)					0.20 (±0.01)		
32.37	<i>epi</i> - <i>α</i> -Muurolol	1649			0.81 (±0.04)							
33.18	<i>α</i> -Bisabolol	1686	0.30 (±0.01)					0.27 (±0.03)				
35.46	Benzyl benzoate	1732		3.61 (±0.08)								
	Monoterpenes hydrocarbons		4.87	0.88	3.54	5.29	0.32	12.56	38.53	96.57	7.88	39.90
	Oxidated monoterpenes		83.23	0.61	47.05	75.24	88.69	86.90	53.65	2.83	89.20	47.42
	Sesquiterpenes hydrocarbons		10.80	75.06	4.59	9.34	9.76	0.34	5.16	0.00	1.70	8.48
	Oxidated sesquiterpenes		0.00	19.05	0.21	6.13	0.44	0.00	0.64	0.00	0.00	1.30
	Others		0.00	0.00	43.81	0.00	0.09	0.00	0.52	0.00	0.32	0.00
	Total identified compounds		98.90	95.60	99.20	96.00	99.30	99.80	98.50	99.40	99.10	97.10

686 Table 2. Repulsion indexes of the tested essential oils, with significant *p*-values highlighted in
 687 bold; n represents the number of spiders tested per oil.

Oil	n	Repulsion Index	t-value	p-value
<i>Catnip</i>	31	0.99	-9.208	<0.0001***
<i>Cedarwood</i>	31	0.83	-3.581	0.001**
<i>Cinnamon</i>	32	0.99	-9.820	<0.0001***
<i>Citronella</i>	29	0.81	-3.585	0.001**
<i>Clove</i>	28	0.99	-6.646	<0.0001***
<i>Eucalyptus</i>	32	0.52	-0.445	0.659
<i>Lavender</i>	31	0.49	0.108	0.915
<i>Lemon</i>	31	0.47	0.829	0.414
<i>Peppermint</i>	33	0.45	0.935	0.357
<i>Tea tree</i>	33	0.56	-1.377	0.178

688