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ABSTRACT

The pea aphid *Acyrthosiphon pisum* Harris is a major insect pest of *Fabaceae* crops causing significant damage and economic losses. Its management is a real challenge and the use of plant essential oils (EOs) as biorepellents is a serious alternative to chemical pesticides. Here, we conducted for the first time an in-depth study on the effects of forty plant EOs and their chemical compounds on *A. pisum*. Using choice bioassays, we reveal the strong repellency of Chinese cinnamon, peppermint, anise, basil, spearmint, and dill oils. Analysis of their chemical composition shows that their respective high contents of trans-cinnamaldehyde, trans-anethole, menthol + menthone, estragole and carvone could be responsible for their high repellency. An additional economic analysis underlines that peppermint EO is the most available and the cheapest. Our data show that peppermint EO with menthol and menthone as major compounds seems to be the most promising EO as biorepellent against the pea aphid. Overall, the repellent activity of peppermint EO may provide a new way to control *A. pisum* in the future, which may lead to effective strategies for controlling these sucking pests.

1. Introduction

The pea aphid *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) is a major pest of *Fabaceae* crops such as peas, broad bean, or alfalfa. It is an early developing species that can rapidly colonize crops due to the long distance travel of alate adults and its asexual mode of reproduction by parthenogenesis which can occur all year round in mild climates (van Emden and Harrington, 2017; Hullé et al., 2020). The pea aphid feeds on plant phloem sap, affecting plant growth, causing flower abortion, reduced grain weight and pod number, and can be a vector of many pathogens such as plant viruses or saprophytic fungi (van Emden and Harrington, 2017; Hullé et al., 2020). Thus, *A. pisum* causes severe damage to agricultural crops and can lead to considerable economic losses.

The most common method of managing pea aphids is to spray chemical pesticides on infested crops (van Emden and Harrington, 2017) but their use is very controversial as they can develop insect resistance to pesticides (Bass et al., 2015), affect non-target organisms such as beneficial insects (van Emden and Harrington, 2017), pollute the environment (van der Werf, 1996) and threaten human health (Alavanja et al., 2004). Thus, several institutions such as the European Union are promoting the reduction of pesticide use and the adoption of Integrated Pest Management (IPM) practices by farmers through the European Greendeal's "farm to fork" strategy (Farm to Fork Strategy, 2023).

The IPM strategy is based on the coordinated use of multiple techniques to keep pest populations below their economic injury level (Radcliffe et al., 2008). A major principle of IPM is to promote a balance between pests and their natural enemies and to use pesticides only as a last resort by favoring natural products with targeted action that preserve the environment.

In this respect, plant natural products, especially essential oils (EOs), have many advantages as they are biodegradable, readily available, proven non-toxic to mammals and effective on specific targets without developing pest resistance due to their complex chemical composition (Khater, 2012; Guleria and Tiku, 2009; Devrnja et al., 2022). Plant EOs are mainly obtained by steam distillation of vegetative parts of plants, such as leaves or flowers. The resulting oil is a complex mixture of volatile organic compounds (VOCs) that are secondary metabolites such as terpenoids, produced by plants, mainly in response to various stresses, including pest attacks (Picazo-Aragonés et al., 2020). The required properties of an EO, whether for use in pharmacology, aromatherapy, the food industry or against agricultural pests, are mainly attributed to its main components and their synergy, and this composition may vary depending on the crop conditions (Dardouri et al., 2019).

Many studies have demonstrated the effectiveness of EOs as biopesticides against various pests, including aphids (Devrnja et al., 2022; Menossi et al., 2021; Atanasova and Leather, 2018; Ikbal and Pavela, 2019). However, in order to preserve the balance between the populations of harmful and beneficial insects on crops and to limit the risks of side effects of EOs which are still not well-known, research is heading towards their use at lower doses as biorepellents (Ndakidemi et al., 2016; Hikal et al., 2017; Toledo et al., 2019; Sayed et al., 2022; Sánchez Chopa and Descamps, 2012). While the repellency of EOs against arthropods has been extensively studied against mosquitoes, research is nowadays being progressively extended to agricultural pests (Nerio et al., 2010; da Silva and Ricci-Júnior, 2020).

Over the last two decades, the repellent effect of EOs on aphids has received increasing attention. A wide range aromatic and medicinal plant EOs have been shown to repel the highly polyphagous peach-potato aphid *Myzus persicae* Sulzer (Castresan et al., 2013; Hori, 1999a; Valcárcel et al., 2021; Dancewicz et al., 2012, 2015; Cantó-Tejero et al., 2022; Khaled-Gasmi et al., 2021; Oulebsir-Mohandkaci and Ait Slimane- Ait Kaki, 2015; Hori, 1998, 1999b). Many of them also have a repellent effect on the bird cherry-oat aphid *Rhopalosiphum padi* L., which feeds on *Poaceae* such as wheat or maize (Valcárcel et al., 2021; Pascual-Villalobos et al., 2017; Grul'ová et al., 2017). Finally, a few have been tested on other aphid species (Sayed et al., 2022; Castresan et al., 2013; Hori, 1999a; Cantó-Tejero et al., 2022; Khaled-Gasmi et al., 2021; Denoirjean et al., 2022; Jiang et al., 2016; Alotaibi et al., 2022; Hosseini Amin et al., 2013). In particular, against the pea aphid *A. pisum*, the EOs of some plants cultivated worldwide, namely anise, caraway, marjoram, oregano, rosemary, summer savory and thyme (Dancewicz et al., 2012), as well as some endemic plants (Kasmi et al., 2017; Zapata et al., 2010; Bruce et al., 2005) have shown promising repellent results. However, further research as well as economic and practical considerations are required for the sustainable development of commercial biorepellents (Isman, 2020).

In this study, we screened forty EOs for their repellency against the pea aphid *A. pisum*. In addition, we analyzed their chemical composition by gas chromatography - mass spectrometry (GC-MS) and related it to their repellent activity to determine possible VOCs involved and conclude with better understanding on a selection of promising EOs. Finally, in order to consider the commercial development of theses EOs as sustainable biorepellents, their bioactivity was crossed with their availability and price.

2. Material and methods

2.1. Aphid rearing

The pea aphids *A. pisum* used in this study were virginogeniae parthenogenetic females from a field originated line LL01. Aphid were reared on young plants of *Vicia faba* L. 'Aquadulce' broad beans under controlled conditions in a growth chamber at 20 ± 2 °C with $65\% \pm 5\%$ relative humidity (RH) and a 16 h light: 8 h dark photoperiod. The lighting consisted of cold white LED tubes and Fluora fluorescent tubes supplied by Philips (Amsterdam, Netherlands) and Osram Lighting (Munich, Germany), respectively. Organic seeds of *V. faba* supplied by Graines-Voltz (Loire-Authion, France) were germinated in commercial peat substrate TRH400 Florentaise (Saint-Mars-du-Désert, France).

The aphids used for the repellency bioassay were synchronized one-day-old apterous nymphs (N1, first instar) from alate adults according to a standard protocol previously described (Simonet et al., 2016).

2.2. Plant essential oils

The forty essential oils used in this study were selected according to their repellent or insecticidal activity reported in the literature against aphids as well as other crop pests or mosquitoes. This includes some oils that have already been tested against the pea aphid, thus consolidating previous results, but also many new oils that have never been tested.

The EOs were provided by different sources in France and come from conventional or organic industrial crops worldwide. All details are presented in Table 1.

2.3. Binary-choice repellency bioassay

The repellency bioassay was carried out for 24 h in a climate chamber at 20 ± 2 °C with $65\% \pm 5\%$ RH in the dark to avoid any possible light bias. N1 aphid nymphs were placed in the center of a tube (10.6 cm x $\varnothing$ 1 cm; 8.33 cm^3) sealed on both sides with an artificial diet coupled to a strip of filter paper impregnated with ethanol for the control side and a mixture of ethanol and EO for the treatment side (Fig. 1). The aphids had to choose by olfaction which direction to go and pass beyond the impregnated strip (with or without contact) before settling on the artificial diet. The next day, the number of settled aphids was counted on each side of the tube. The test was repeated 18 times with 5 aphids per tube, for a total of 90 aphids.

First instar nymphs were favored for their ability to feed on an artificial diet, which allowed these initial tests to be carried out under in vitro conditions without plant interaction. The complete nutritive artificial diet used in this study was specifically developed for *A. pisum* feeding and development (Febvay et al., 1988). The filter paper strips were previously impregnated with 10 µl of pure ethanol solution with or without EO and then dried by evaporation. Knowing that research in biopesticides is moving towards biopolymer materials loaded with EO for a controlled release of VOCs with lower environmental impact (Menossi et al., 2021), we calculated the dose of essential oil to be applied according to a weight concentration of the paper strip. Thus, for the forty EOs, we applied a minimum dose of 1% w/w (0.12 µl EO at 0.94 average density (Porter and Lammerink, 1994) using 10 µl EO solution at 1,2% v/v), also corresponding to 0.08 µl/cm^2 of filter paper or 0.014 µl/cm^3 in the tube.

A count of aphids on the artificial diet or within 1 cm was carried out on each side of the tubes as well as a careful assessment of aphids' condition. A minimum threshold of 60% settled aphid was established for data analysis with solid conclusions. A low settlement rate with agitated, underweight, or dead aphids was recognized as a toxic response to EOs of this bioassay. EOs that were found to be toxic were excluded from further data analysis of repellent activity. Finally, a Repellency Index (%) (RI) of the remaining EOs was calculated according to the following formula (McDonald et al., 1970):

Table 1

List of the forty plant essential oils tested.

Scientific name	Family	Common name	Plant organ	Production	Country	Source
Allium sativum L.	*Amaryllidaceae*	Garlic	Bulb	Organic	India	Aroma-Zone (Paris, France)
Anethum graveolens L.	*Apiaceae*	Dill	Seed	Conventional	Hungary	Voshuiles (Nevers, France)
Carum carvi L.	*Apiaceae*	Caraway	Seed	Conventional	Hungary	Voshuiles (Nevers, France)
Cinnamomum camphora (L.) J. Presl	*Lauraceae*	Camphor tree	Bark	Conventional	Central Asia	Voshuiles (Nevers, France)
Cinnamomum cassia (L.) J. Presl	*Lauraceae*	Chinese cinnamon	Aerial parts	Organic	China	Voshuiles (Nevers, France)
Citrus aurantium L.	*Rutaceae*	Bitter orange	Leaf	Organic	Paraguay	Voshuiles (Nevers, France)
Citrus limon (L.) Burm. f.	*Rutaceae*	Lemon	Zest	Organic	Sicily	Aroma-Zone (Paris, France)
Citrus sinensis (L.) Osbeck	*Rutaceae*	Orange	Zest	Organic	Mexico	Voshuiles (Nevers, France)
Coriandrum sativum L.	*Apiaceae*	Coriander	Seed	Conventional	Moldova	Voshuiles (Nevers, France)
Corymbia citriodora (Hook.) K.D.Hill & L.A.S.Johnson	*Myrtaceae*	Lemon-scented gum	Leaf	Organic	Madagascar	Aroma-Zone (Paris, France)
Cuminum cyminum L.	*Apiaceae*	Cumin	Seed	Conventional	India	Voshuiles (Nevers, France)
Curcuma longa L.	*Zingiberaceae*	Turmeric	Rhizome	Organic	India	Voshuiles (Nevers, France)
Cymbopogon winterianus L.	*Poaceae*	Java citronella	Leaf	Conventional	Indonesia	Voshuiles (Nevers, France)
Eucalyptus globulus Labill.	*Myrtaceae*	Southern blue gum	Leaf	Organic	Portugal	La Drôme Provençale SA (Saillans, France)
Foeniculum vulgare Mill.	*Apiaceae*	Fennel	Seed	Organic	Hungary	Aroma-Zone (Paris, France)
Laurus nobilis L.	*Lauraceae*	Bay laurel	Leaf	Organic	Turkey	Voshuiles (Nevers, France)
Lavandula angustifolia Mill.	*Lamiaceae*	True lavender	Flowering top	Organic	France	Voshuiles (Nevers, France)
Lavandula officinalis Mill.	*Lamiaceae*	Officinal lavender	Flowering top	Organic	Bulgaria	La Compagnie des Sens (Lyon, France)
Melaleuca alternifolia (Maiden & Betche) Cheel	*Myrtaceae*	Tea tree	Leaf	Organic	Australia	Phytosun arôms - Omega Pharma (Châtillon, France)
Mentha piperita L.	*Lamiaceae*	Peppermint	Aerial parts	Organic	India	Voshuiles (Nevers, France)
Mentha pulegium L.	*Lamiaceae*	Pennyroyal	Aerial parts	Conventional	Morocco	Pranarôm (Lille, France)
Mentha spicata L.	*Lamiaceae*	Spearmint	Flowering top	Organic	India	Aroma-Zone (Paris, France)
Myrtus communis L.	*Myrtaceae*	Common myrtle	Aerial parts	Conventional	Tunisia	Voshuiles (Nevers, France)
Ocimum basilicum L.	*Lamiaceae*	Basil	Flowering aerial parts	Organic	India	La Compagnie des Sens (Lyon, France)
Origanum compactum L.	*Lamiaceae*	Compact oregano	Whole flowering plant	Organic	Morocco	Voshuiles (Nevers, France)
Origanum majorana L.	*Lamiaceae*	Marjoram	Aerial parts	Organic	Egypt	Aroma-Zone (Paris, France)
Origanum vulgare L.	*Lamiaceae*	Oregano	Aerial parts	Organic	Spain	Voshuiles (Nevers, France)
Pelargonium graveolens L'Hér.	*Geraniaceae*	Rose geranium	Aerial parts	Organic	Egypt	Voshuiles (Nevers, France)
Petroselinum crispum (Mill.) Fuss	*Apiaceae*	Parsley	Seed	Conventional	France	Voshuiles (Nevers, France)
Pimenta dioica (L.) Merr.	*Myrtaceae*	Allspice	Leaf	Conventional	Jamaica	Voshuiles (Nevers, France)
Pimpinella anisum L.	*Apiaceae*	Anise	Seed	Conventional	Spain	Aroma-Zone (Paris, France)
Piper nigrum L.	*Piperaceae*	Black pepper	Unripe drupe	Conventional	India	Voshuiles (Nevers, France)
Pogostemon cablin (Blanco) Benth.	*Lamiaceae*	Patchouli	Leaf	Organic	Madagascar	Voshuiles (Nevers, France)
Salvia officinalis L.	*Lamiaceae*	Common sage	Flowering top	Organic	Albania	Pranarôm (Lille, France)
Salvia rosmarinus CT cineole Spenn.	*Lamiaceae*	Rosemary CT cineole	Flowering top	Organic	Morocco	Voshuiles (Nevers, France)
Salvia rosmarinus CT verbenone Spenn.	*Lamiaceae*	Rosemary CT verbenone	Flowering aerial parts	Organic	South Africa	Voshuiles (Nevers, France)
Salvia sclarea L.	*Lamiaceae*	Clary sage	Flowering aerial parts	Organic	Spain	Voshuiles (Nevers, France)
Satureja montana L.	*Lamiaceae*	Winter savory	Aerial parts	Conventional	Balkans	Voshuiles (Nevers, France)
Syzygium aromaticum (L.) Merr. & L.M. Perry	*Myrtaceae*	Clove	Flower bud	Organic	Madagascar	Aroma-Zone (Paris, France)
Thymus vulgaris CT thymol L.	*Lamiaceae*	Thyme CT thymol	Flowering top	Conventional	Spain	Voshuiles (Nevers, France)
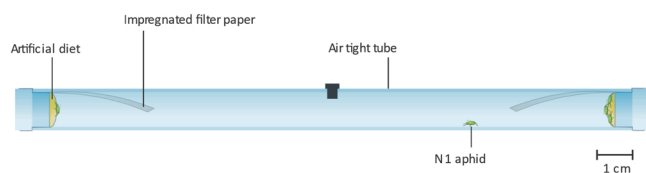
Fig. 1. Binary-choice bioassay tube for aphid repellency. Groups of 5 aphids are introduced in the center of the tube and have one day to choose between the control side with ethanol-impregnated filter paper and the treatment side with a mixture of ethanol and essential oil and to settle on the artificial diet.

$$RI = [(C - T)/(C + T)] \times 100$$

Where C is the total number of aphids on the control side, and T is the total number of aphids on the treatment side. Positive and negative values indicate repellent and attractant effects, respectively. Next, for a better understanding and discussion of the results of this bioassay, the RI of EOs was grouped in relative repellency classes according to the classification presented in Table 2, adapted from McDonald et al (McDonald et al., 1970).

2.4. Statistical analysis

To compare the repellent activity of EOs according to the total number of aphids settled on the control side and the treatment side, we

Table 2

Relative repellency classes of essential oils (EOs).

Classes	Repellency Index (%)	Property of the EO
Class V	80.1 to 100	Very repellent
Class IV	60.1 to 80	Repellent
Class III	40.1 to 60	Moderately repellent
Class II	20.1 to 40	Weakly repellent
Class I	0.1 to 20	Very weakly repellent
Class 0	0	Neutral
Class -I	−0.1 to −20	Very weakly attractive
Class -II	−20.1 to −40	Weakly attractive
Class -III	−40.1 to −60	Moderately attractive
Class -IV	−60.1 to −80	Attractive
Class -V	−80.1 to −100	Very attractive

performed a pairwise chi-squared test (Siegel and Castellan, 1988) for each pair of EOs, and applied a p-value correction by FDR-Benjamini-Hochberg multiple testing (Benjamini and Hochberg, 1995). The chi-squared test was executed using the SciPy Python library (Virtanen et al., 2020), and the multiple-test p-value correction was run using the Statsmodels Python library (Seabold and Perktold, 2010).

2.5. Chemical analysis of plant essential oils

The chemical composition of the forty EOs was determined by GC-MS using an HP 6890 GC system connected to an HP 5973 Mass Selective Detector equipped with a DB-5 MS capillary column (60 m x 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies, Palo Alto, CA, USA).

The EOs were diluted in hexane (1:100) and 1 μl was injected. The column temperature was held at 60 °C for 2 min, programmed to increase at 6 °C/min to 220 °C and then held at this temperature for 2 min. Hydrogen was used as carrier gas with a flow rate 2.8 ml/min and a split ratio of 20:1. The temperature of the injector was maintained at 210 °C. The MS was set to scan in the range of m/z 35–500 amu with an ionization energy set to 70 eV. The quadrupole temperature was kept at 150 °C and the ion source temperature at 230 °C.

The chemical components of EOs were identified by comparison of their retention indices and MS spectra with those reported in the literature (Adams, 2007; Linstrom and Chemistry, 1997) and using Masshunter Qualitative Analysis software (B07.00, Agilent Technologies, USA) matching with standard reference databases (NIST11, Wiley275 and CNRS libraries). The retention index of each component was calculated relative to a standard mix of n-alkanes (C7-C26, Sigma-Aldrich, St. Louis, MO, USA), analyzed under identical experimental conditions. The concentration of the identified components was computed based on the percentage of their relative peak area (%). A heatmap providing the list and content of all chemical compounds found in the forty EOs at a relative percentage of at least 1% and their respective retention index is available in Fig. 4.

2.6. Chemical composition related to repellent activity

To identify possible VOCs involved in repellent activity, we trained a machine learning model, based on the chemical composition of non-toxic EOs and their RI, which is able to classify the compounds according to their impact on the RI. The matrix of EOs compounds represents 34 EOs described by 91 compounds ≥ 1% (Fig. 4). Since some compounds are only present altogether in specific EOs and because the matrix has more features than object, which is not the best fit for the model, we decided to reduce the dimensionality of the dataset by clustering compounds with very similar appearance vectors, so their impact on the RI will be considered as a group.

The EOs compounds were clustered using a hierarchical clustering algorithm on the matrix, based on the complete grouping method and the cosine similarity (Sokal, 1958). By analyzing the clustering distance curve and the dendrogram (Supplementary Fig. S1) associated with clustering, we set a clustering distance threshold of 0.075, forming 47 rather small clusters with compounds showing very similar appearance vectors in the EOs (Supplementary Table S1). The chemical composition of each EO is then described in terms of compound clusters as the sum of the presence of each compound belonging to the cluster.

In order to analyze the impact of each cluster of compounds on the RI of EOs, we trained a RBF-kernel SVM Regressor (Chang and Lin, 2011) to model the RI as a function of the composition of the EO in logarithmic scale (similar results were obtained using a Random Forest Regressor (Breiman, 2001) and a ElasticNet Linear Regressor (Friedman et al., 2010)). The SVM Regressor settings are detailed in Supplementary data with the Fig. S2. Then, we used a Kernel Shapley values Explainer (Lundberg and Lee, 2017) to measure the contribution of each compound cluster in the RI regression task, for each EO. The 9 most

determinant clusters per EO (plus the contribution of the remaining clusters) are shown in Supplementary Fig. S3. Finally, we computed the 25 most determinant clusters of compounds on the RI as a function of their abundance, for all EOs, and cross-referenced these findings with biological information available in the literature. The SciPy python library was used to perform the clustering, the Scikit learn library (Pedregosa et al. n.d.) was used to train the SVR regressor, and the SHAP library was used to compute Shapley values.

2.7. Economic analysis

To consider the commercial development of the most promising EOs as biorepellents, their bioactivity was cross-referenced with their availability and price. The most repellent EOs corresponding to classes V and IV were selected. The bioactivity of the EOs was expressed by their respective RI. Then, the availability and price of EOs were determined for conventional and organic production respectively. Their availability was represented by the number of producing countries estimated from the global export records from the intelligence company Volza (Rehoboth Beach, DE, USA). These data were retrieved online[5] using the following keywords: "*Product HS code*" + "*Product name*" (if no specific HS code) +" organic" (for organic data). Finally, as the average price of EOs can vary greatly from one country to another, we were interested on the price in France as an example where the development of EO-based biorepellents could be part of the Ecophyto II+ plan (Le Gouvernement, 2015). For that purpose, we computed the average price as of November 17, 2022 of EOs (ex-tax €/l) from three major French suppliers, including two wholesalers (Stever, Mâcon; Madatrano, Sainte-Pazanne) and a leading company specializing in the online sale of EOs at low prices (Aroma-Zone, Paris)[5]. Prices were obtained on request from Stever's sales department and from the online catalogs of Madatrano and Aroma-zone.[6]

3. Results and discussion

3.1. Repellent activity

The repellent activity of the EOs against the pea aphid under the conditions of the bioassay was clustered according to their RI in Fig. 2 for a better understanding and discussion of the results. The six EOs of pennyroyal (*Mentha pulegium*), patchouli (*Pogostemon cablin*), oregano (*Origanum vulgare*), parsley (*Petroselinum crispum*), compact oregano (*Origanum compactum*), and thyme (*Thymus vulgaris*) CT thymol were found to be toxic in this bioassay at the tested concentration. Considering the potential incorporation of EOs into biopolymer materials for a controlled release of VOCs with lower environmental impact, they were removed. If the toxicity of these EOs is mainly dependent on the dose applied and not intrinsic to their composition, additional investigations could reveal their repellent potential against the pea aphid at much lower dose. The other EOs showed very variable RI ranging from class V, very repellent, to class -III, moderately attractive.

P-values associated to the statistical comparison between each pair of EOs are represented as a heatmap in Fig. 3. At first sight, the p-values heatmap highlights groups of EOs that are not significantly different from each other as follows: class V and IV (very repellent and repellent), class III (moderately repellent), classes II and I (weakly and very weakly repellent), classes -I and -II (very weakly and weakly attractive), then class -III (moderately attractive). Between these groups, class V Chinese

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[5] Volza.com - Global Export Import Trade Data of 209 Countries, (n.d.). https://www.volza.com/ (accessed November 17, 2022).

[6] Huiles Essentielles et Huiles Végétales de Madagascar et du monde entier, (n.d.). https://www.madatrano.com/ (accessed November 17, 2022). and Aroma-Zone – Huiles essentielles, Beauté Nature et Cosmétique maison, (n.d.). https://www.aroma-zone.com/ (accessed November 17, 2022).
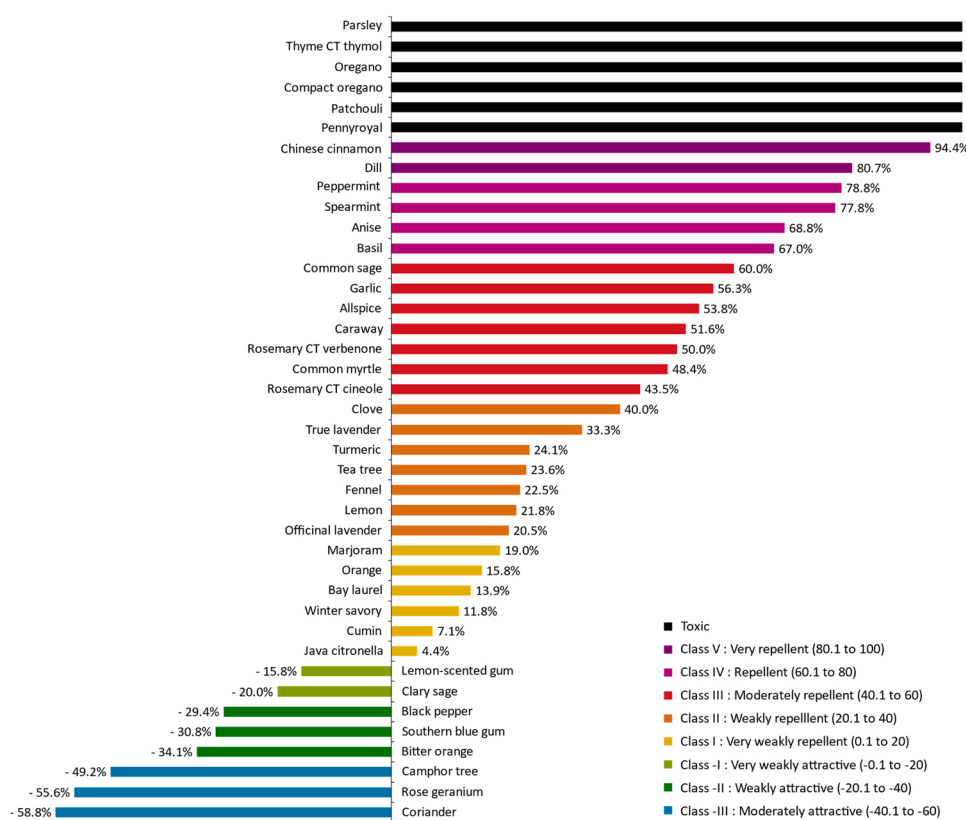
Fig. 2. Repellency index (RI) of forty essential oils against *Acyrthosiphon pisum* in binary-choice bioassays after 24 h at a dose of 1% w/w corresponding to 0.014 μl/cm^3 in the tube. RI (%) = [(C − T)/(C + T)] × 100 where C is the total number of aphids on the control side, and T is the total number of aphids on the treatment side. Positive and negative values indicate repellent and attractant effects, respectively. EOs are grouped in relative repellency classes for guidance. Some EOs were found to be toxic under the conditions of this bioassay.

cinnamon (*Cinnamomum cassia*) oil is the most repellent against the pea aphid (94.4%) and significantly different from all other EOs while class V dill (*Anethum graveolens*) oil (80.7%) is virtually not different from class IV EOs i.e. peppermint (*Mentha piperita*), spearmint (*Mentha spicata*), anise (*Pimpinella anisum*), and basil (*Ocimum basilicum*) (78.8%, 77.8%, 68.8% and 67%, respectively). Furthermore, class IV basil oil is not different from class III common sage (*Salvia officinalis*), garlic (*Allium sativum*), allspice (*Pimenta dioica*), caraway (*Carum carvi*), and rosemary (*Salvia rosmarinus*) CT verbenone oils (60%, 56.3%, 53.8%, 51.6% and 50%, respectively).

The EOs showing the strongest significant activity were compared with the results from previous studies on *A. pisum* as well as on other aphids, to investigate if some of these oils would have a repellent effect on a wider range of aphids (Table 3). This comparison of the different test conditions highlights that these studies mainly use adult aphids and EO impregnated leaves while our bioassay used N1 nymphs without plant interaction.

For instance, former study on *A. pisum* using a 0.1% EO solution concluded that oregano and caraway oils were repellent (70%), rosemary oil was weakly repellent (40%), and anise and thyme oils were very weakly repellent (20% and 8% respectively) (Dancewicz et al., 2012). In our bioassay using a 1.2% EO solution, oregano and thyme CT thymol oils were toxic, anise oil is repellent (68.8%), and caraway and rosemary CT verbenone/cineole were moderately repellent (51.6%, 50% and 43.5% respectively). With a twelve times solution more concentrated, these EOs have overall a stronger bioactivity, more or less increasing according to the EO. However, this is not the case for caraway oil whose RI is lower in this bioassay, which means that other factors such as the stage of aphid and the interaction with the plant may play a role in the repellency of EOs. This was investigated in a recent study, showing that the interaction with the plant made the repellency of garlic oil insignificant on the rosy apple aphid *Dysaphis plantaginea* (Passerini) (Denoirjean et al., 2022). However, while the stage of the aphid may play a role in EOs repellency, the apterous or alate form of the aphid

does not appear to make a significant difference in EO bioactivity according to other research on the rose-grain aphid *Metopolophium dirhodum* (Walk.) using rosemary oil (Sánchez Chopa and Descamps, 2012). Thus, future works would aim to extend research on the most repellent oils against *A. pisum* at different doses on later stages and with interaction with the plant to consider their use as a biorepellent against a pea aphid population on *Fabaceae* crops. More specifically, because the repellency is related to a change in insect foraging behavior, it would be important to perform additional no-choice and choice tests evaluating aphids feeding behavior on plants, as in Denoirjean et al (Denoirjean et al., 2022)., thus challenging the conclusions of this laboratory study under conditions closer to the field. Indeed, the transition from laboratory to realistic agronomic conditions may affect the efficiency of EOs in controlling pest populations (Dunan et al., 2021). For instance, VOCs emitted by the host plant and potential lack of surrounding alternative food sources could impair the repellent effect of EOs under field conditions.

Regarding the other aphid species, the EOs listed in Table 3 that have a toxic, repellent, or attractive effect on the pea aphid all have a repellent effect on other aphids, also becoming stronger as the dose increases. Indeed, at doses close to ours, toxic EOs against the pea aphid were weakly repellent against *R. padi* for pennyroyal (Pascual-Villalobos et al., 2017), repellent for oregano (Valcárcel et al., 2021) and very repellent for thyme (Valcárcel et al., 2021; Grul'ová et al., 2017) against both *M. persicae* and *R. padi*, and the attractive coriander (*Coriandrum sativum*) oil on the pea aphid was very repellent, repellent, and weakly repellent against *M. persicae*, the potato aphid *Macrosiphum euphorbiae* (Thomas) (Cantó-Tejero et al., 2022), and *R. padi* (Pascual-Villalobos et al., 2017), respectively. Thus, an oil that is toxic or attractive in one aphid species can have a very different effect in another species, which supports the need to search for oils that are effective in priority against a specific target, such as *A. pisum*.

As for EOs being very effective as biorepellent against *A. pisum*, research on the polyphagous aphids *M. persicae* and *M. euphorbiae* using
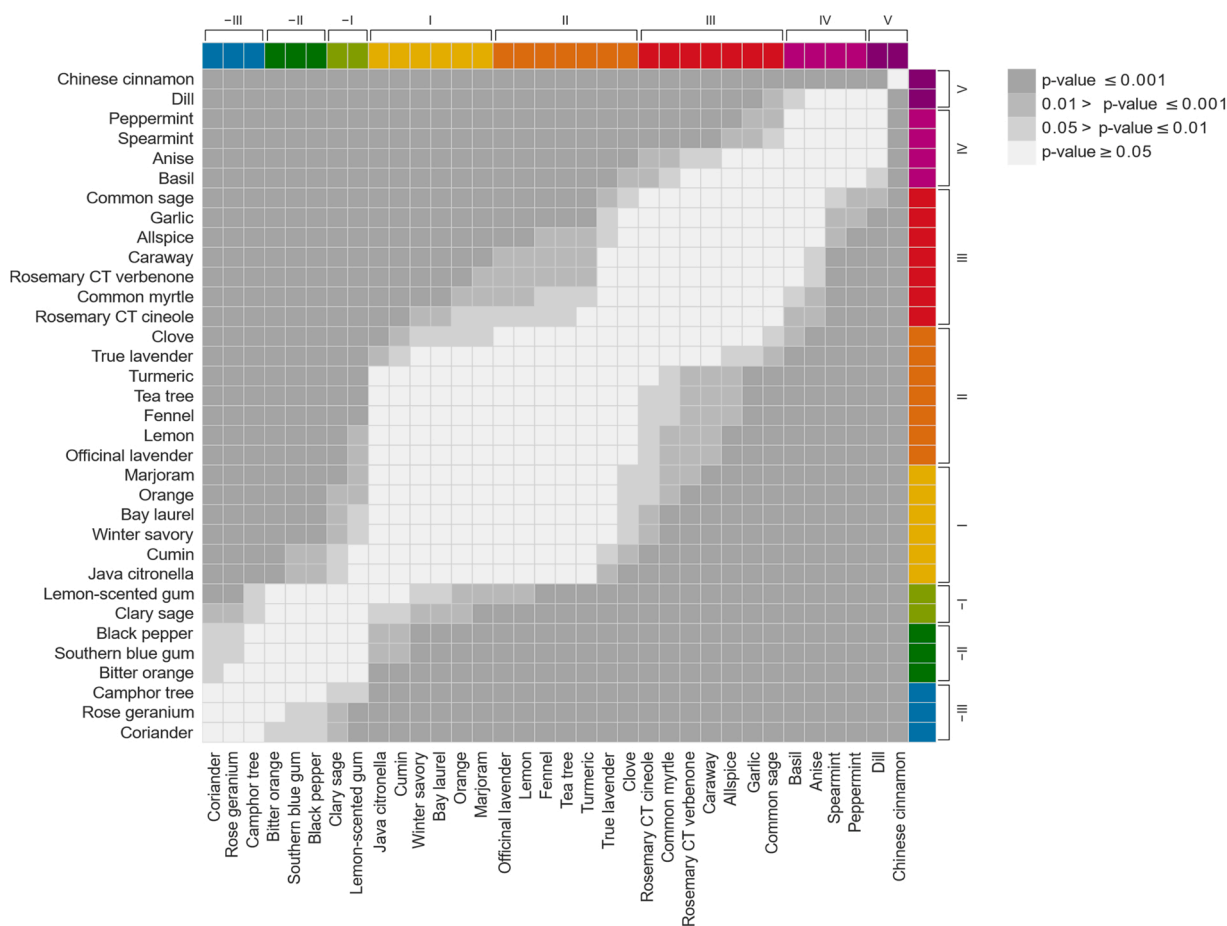
Fig. 3. Heatmap representing the level of significance of the p-values of pairwise chi-squared tests comparing the repellent activity of essential oils. The oils are organised by relative repellency classes under bioassay conditions ranging from V (very repellent) to -III (moderately attractive).

a ventilated device and doses comparable to ours (Valcárcel et al., 2021; Cantó-Tejero et al., 2022) showed results close to those obtained on the pea aphid. Specifically, the peppermint, anise and basil oils that are repellent in this study were found to be repellent and moderately repellent with respect to peppermint for *M. persicae* and *M. euphorbiae*, respectively, and very repellent regarding anise and basil for both aphids *(Cantó-Tejero et al., 2022)*. Another study using the same EOs at a slightly lower dose on *R. padi* feeding on *Poaceae* demonstrated that they were moderately repellent in a ventilated device and became repellent and very repellent for peppermint and anise, respectively, in an air tight device (Pascual-Villalobos et al., 2017). These results indicate that peppermint, anise and basil oils, in addition to being repellent to the pea aphid, could also be effective against a polyphagous aphid attack on *Fabaceae* as well as oligophagous aphids on other crops in the vicinity or in a rotation.

3.2. Chemical composition

The difference in aphids' responses to EOs could be attributed to the differences in their chemical composition, presented as a heatmap in Fig. 4. Indeed, aphids are able to perceive the different plant VOCs through their antennae equipped with olfactory receptor neurons that will activate a signaling cascade and induce a behavioral response adapted to the odor: flight, foraging, etc (Pickett et al., 1992). Plant VOCs are mainly terpenoids such as monoterpenes and sesquiterpenes biosynthesized via the methyl erythritol phosphate and the mevalonate pathways, and, to a lesser extent, phenylpropanoids derived from the shikimate pathway (Picazo-Aragonés et al., 2020; Regnault-Roger et al., 2012).

The major components of the six EOs found to be toxic on *A. pisum* are myristicin and apiol in parsley (phenylpropanoids), patchouli alcohol in patchouli (oxygenated sesquiterpene), thymol in thyme CT thymol, carvacrol in oregano and compact oregano, and pulegone in pennyroyal (oxygenated monoterpenes). All of these compounds are known as neurotoxic to insects, inhibiting neurotransmitters such as acetylcholinesterase and disrupting the functioning of GABA and octopamine synapses, making it challenging to consider their use as biorepellent at lower dose (Ikbal and Pavela, 2019; Regnault-Roger et al., 2012; Achimón et al., 2022; Zhu et al., 2003; Park and Tak, 2016).

Regarding the 34 remaining EOs, their chemical composition (Fig. 4) was divided into clusters of compounds for the analysis of their impact on the RI, in order to consider the effect of compounds that appear altogether and not only the content of the main compounds (Supplementary Table S1). Thus, we trained a machine learning model capable of evaluating the impact of each cluster of compounds on EOs repellency and to model a RI for each EO based on their composition, having a strong match with the RI obtained during the bioassay (Supplementary Fig. S3). According to the model calculations, some clusters of compounds did not have a significant impact on the modeled RI while other clusters of compounds had a strong negative or positive impact on the modeled RI. Then, to reveal these clusters of interest, we classified the compounds clusters of all EOs according to their impact on the modeled RI and computed the 25 most determinant clusters in Fig. 5. Some clusters are composed of different chemical families because the model found them altogether in a single EO. A larger database would allow to refine the formation of clusters by the model. Here, we have cross-referenced these compound clusters with those in the literature and highlighted the compounds with a predominant role in the bioactivity of

Table 3
Comparison of the essential oils (EOs) showing the strongest significant activity in this study with previous studies on *A. pisum* as well as other aphids.

		A. pisum Our study	*M. persicae* (Dancewicz et al., 2012)	*M. persicae* (Valcárcel et al., 2021)	*M. euphorbiae*[a] (Cantó-Tejero et al., 2022)	*R. padi* (Valcárcel et al., 2021)	*R. padi* (Grul'ová et al., 2017)	*R. padi* (Pascual-Villalobos et al., 2017)	*M. dirhodum*[b] (Sánchez Chopa and Descamps, 2012)	*D. plantaginea*[c] (Denoirjean et al., 2022)
Host plant		*Fabaceae*	Polyphagous			*Poaceae*				*Rosaceae*
Time		24 h								5 mn
EO interaction		Olfaction/contact	Olfaction/contact/ingestion							Olfaction/contact
Medium		Filter paper	Leaf							Cup · Cup+plant
Device		Linear tube	Petri dish							Bridge between cups
Ventilation		Air tight			Air vent			Air tight		Air vent
Aphid form		Apterous							Alate	Apterous
Aphid stage		N1	Adult					N3	Adult	
Dose	EO solution (% v/v)	1.2	0.1	~1.1 *	2	~1.1	~1.1	1.5	< 2.5	~5.3
	EO solution (µl)	10	Dipped leaf	10	10	10	10	10	Dipped leaf	100
	EO (µl)	0.12	/	0.11	0.20	0.11	0.11	0.15	/	5.32
	EO (µl/cm3)	0.014	/	/	0.015	/	0.001	0.011	/	/
EO effect (% RI)	Pennyroyal	Toxic						WR · VWR		
	Oregano	Toxic	70 R · NS			R				
	Thyme	Toxic	8 VWR	61 R	VR	VR	VR			
	Peppermint	78.8 R**		WR	R · MR	WR			MR · R	
	Spearmint	77.8 R		MR		VWR				
	Anise	68.8 R	20 VWR	33 WR	MR	VR	VWR		MR · VR	
	Basil	67 R			VR · VR				MR · MR	
	Garlic	56.3 MR								R · NS
	Caraway	51.6 MR	70 R · NS							
	Rosemary	50 MR	40 WR · NS	MR		VWR			33.3 WR · NS vs alate · 66.7 R · NS vs apterous	
	Coriander	-58.8 MA			VR · R				WR · WR	

[a] Potato aphid *Macrosiphum euphorbiae* Thomas, [b] rose-grain aphid *Metopolophium dirhodum* Walk. and [c] rosy apple aphid *Dysaphis plantaginea* Passerini.

* Conversion of a w/v dose to a v/v dose based on an EO average density of 0.94.

** Very repellent (VR), repellent (R), moderately repellent (MR), weakly repellent (WR), very weakly repellent (VWR), moderately attractive (MA) and not significant (NS) under bioassay conditions.
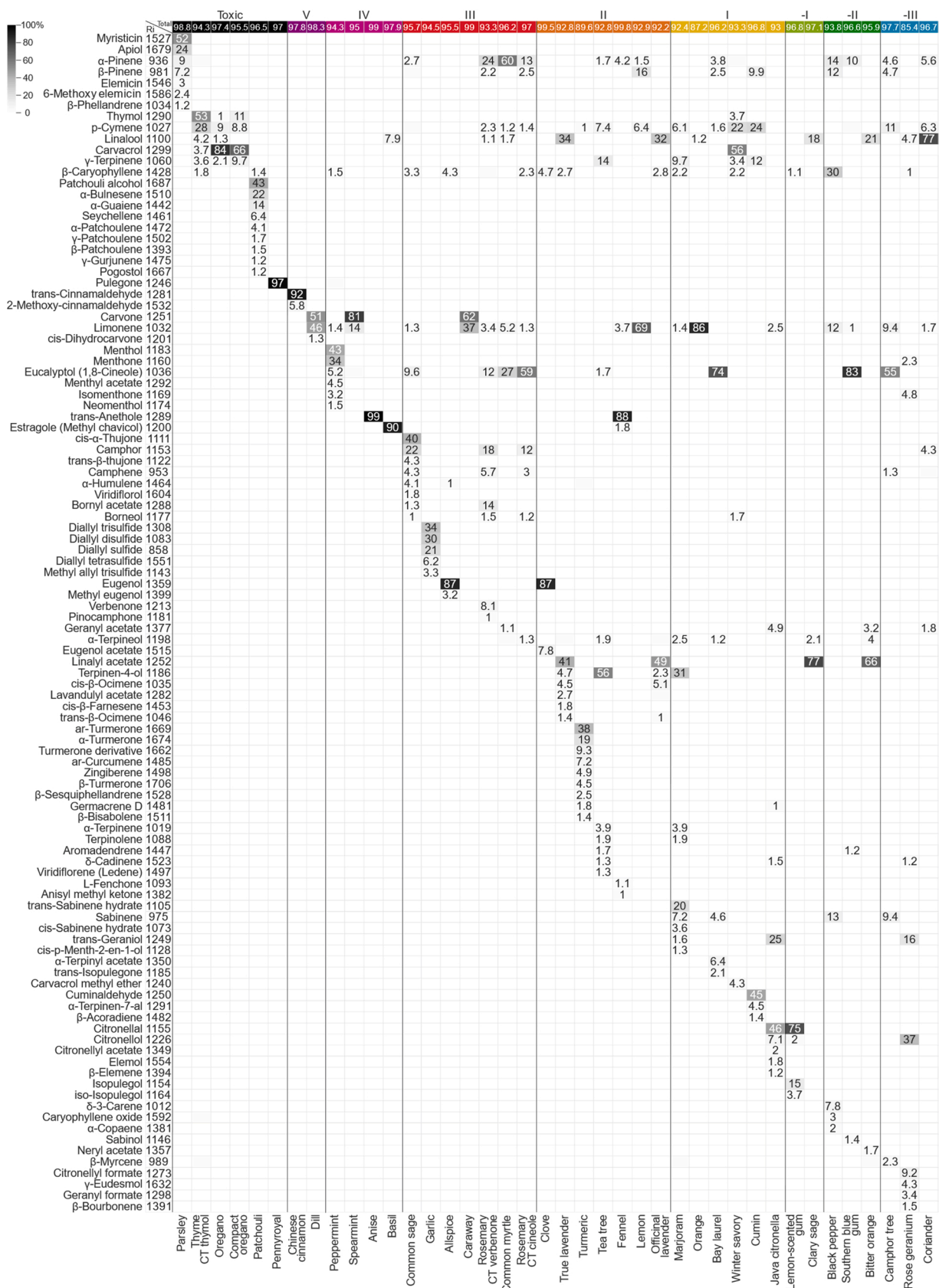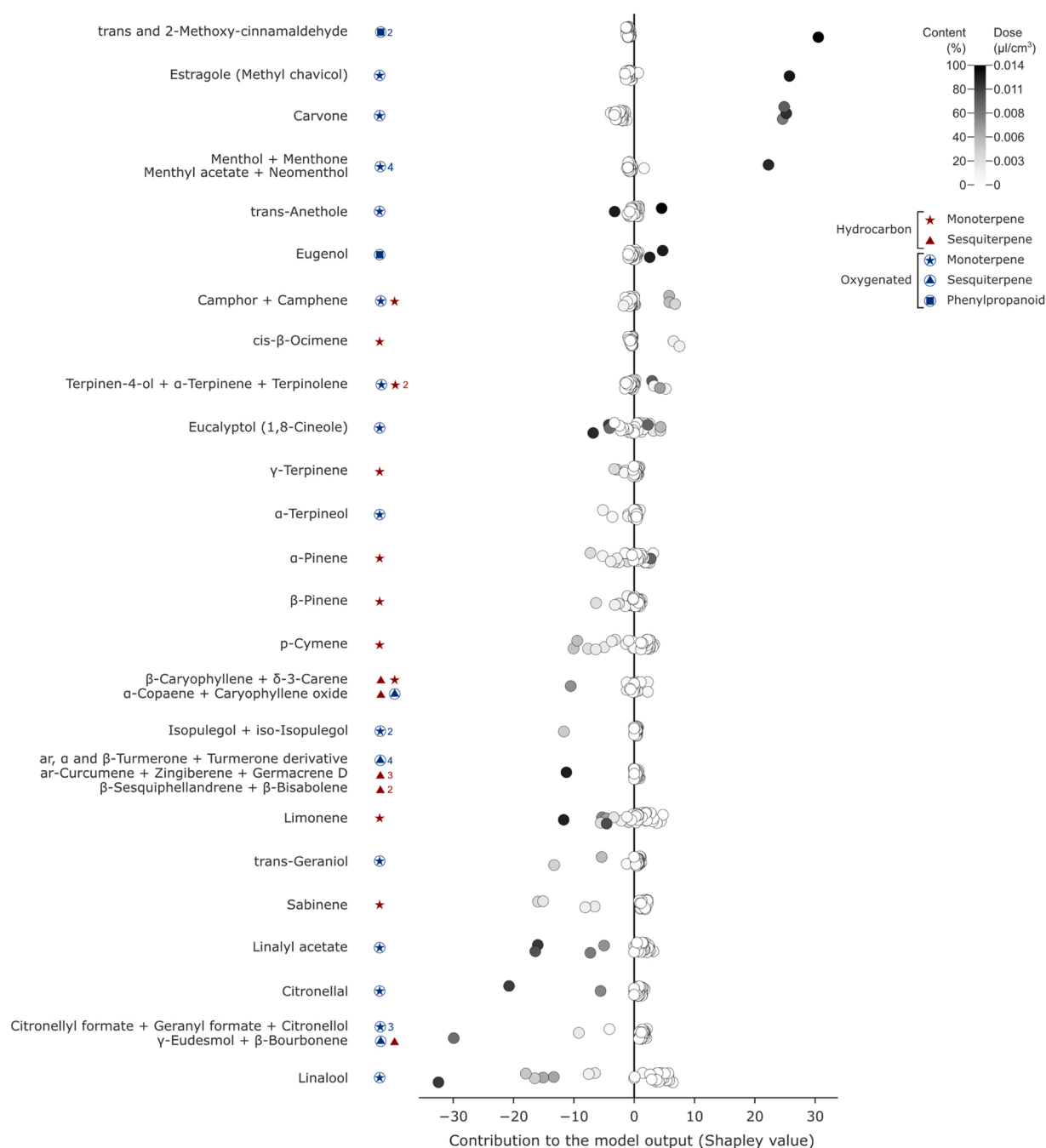
Fig. 4. Heatmap in a grey scale of the relative percentage of the chemical compounds identified in the forty essential oils (EOs) with a threshold of 1% (GC–MS). Data are sorted in decreasing order of percentage one oil after another for compounds, and repellency with corresponding classes according to Table 2 for EOs. Ri = Retention index.

Fig. 5. Clusters of the essential oils (EOs) compounds most determinant for the repellency index (RI) as assessed by the machine learning model. For each cluster, the points represent the 34 EOs resulted as non-toxic against *A. pisum*. The grey scale of the points indicates the content (relative percentage) of the cluster in the EO. The contribute of the clusters (−100 to 100%) to the RI model output for each EO RI is reported as Shapley value. Some clusters are composed of different chemical families because the model found them altogether in only one EO.

EOs.

According to the model calculations, the clusters of compounds with the most positive impact on the modeled RI of EOs against *A. pisum* are also the main components of the most repellent EOs in the bioassay. Thus, based on the conclusions of the model, high contents of trans and 2-methoxy-cinnamaldehyde in Chinese cinnamon, carvone in dill and spearmint, menthol + menthone + menthyl acetate + neomenthol in peppermint, and estragole in basil could be responsible of the high repellency of these EOs found in the bioassay. Trans-anethole in anise and eugenol in allspice also have a positive impact on the modeled RI but to much lesser extent, probably because they were also found in the weakly repellent EOs of fennel and clove. These oxygenated

monoterpenes and phenylpropanoid (particularly trans-cinnamaldehyde, menthol and menthone in their respective clusters) are also known for their relative toxicity and used as active ingredients in biopesticides (Ikbal and Pavela, 2019; Regnault-Roger et al., 2012; Park and Tak, 2016; Koul et al., 2008). Focusing on the pea aphid, a previous study showed that peppermint oil with high menthol and menthone content was very effective as a biopesticide against *A. pisum* (Kimbaris et al., 2010). In addition, research has shown that pea aphids are able to modulate the biosynthesis of metabolites in their host plants and therefore significantly reduce the level of eugenol, which could be a defense strategy against this toxic compound (Sanchez-Arcos, 2018). Thus, all these compounds have great potential as biorepellents against

the pea aphid, provided they are used at very low dose as in our bioassay (close to 0.014 μl/cm^3) to avoid toxic effects.

In addition, camphor (mainly) + camphene present in common sage and rosemary, cis-β-ocimene in lavenders (*Lavandula angustifolia* and *L. officinalis*), terpinen-4-ol (predominantly) + α-terpinene + terpinolene in tea tree (*Melaleuca alternifolia*) and marjoram (*Origanum majorana*), and a slight content of eucalyptol as in common myrtle (*Myrtus communis*), seem to have a small positive impact on the modeled RI (Figs.4–5). According to the literature, levels of terpinen-4-ol in host plants can also be reduced by *A. pisum* as a defense strategy (Sanchez-Arcos, 2018), and a long exposure to a high dose of (*Z*)-ocimene can inhibit their reproduction (Tomova et al., 2005). Finally, a small dose of eucalyptol used in a 3-day fumigant toxicity test was effective against the pea aphid (Attia et al., 2016). These compounds seem to be damaging and could therefore contribute to the repellent activity of EOs against *A. pisum* with the right doses. In this respect, according to the model calculations (Fig. 5), high doses of eucalyptol seem to have a slight opposite effect on the modeled RI, as in the attractive marjoram oil (83% content). Thus, the effects of plant VOCs on aphids are not always become stronger as the dose increases, and this should be considered when choosing a biorepellent composition and dosage.

The remaining most discriminant clusters of compounds on the model have a negative impact on the modeled RI of EOs (Fig. 5), from very low (γ-terpinene) to high (linalool). In the literature, a common assumption is that oligophagous aphids are attracted by the compounds characteristic of their host plants (Dancewicz et al., 2012). Linalool, germacrene D and caryophyllene are precisely present in *V. faba* and attracted the black bean aphid *Aphis fabae* Scopoli in a choice bioassay (Sanchez-Arcos, 2018; Webster et al., 2008). Regarding *A. pisum* also feeding on *V. faba*, linalool and germacrene D were found to inhibit the repellent effect of the alarm pheromone for aphids, (*E*)-β-farnesene (Bruce et al., 2005). Linalool, linalyl acetate and β-pinene also applied against *A. pisum* in a 3-day fumigant toxicity test showed virtually no effect (Attia et al., 2016). Finally, the EO of *Schinus molle* composed of ᴅ-limonene among the major compounds but also of α-caryophyllene, caryophyllene oxide, α-copaene, α-pinene, p-cymene, germacrene D, and γ-eudesmol in low content had an attractive effect on the pea aphid in a choice test, attributed in particular to the presence of limonene and caryophyllene (Kasmi et al., 2017). All these findings may corroborate the negative impact of these clusters of compounds on the modeled RI of an EO against *A. pisum* at high doses (Fig. 5).

Overall, it appears that the clusters of compounds with the most positive or negative impact on the modeled RI of EOs are all oxygenated compounds (Fig. 5). Several studies have already noted this disparity and assumed that this higher efficacy compared to hydrocarbons might be associated with their better lipophilic properties that favor a good penetration of the insect cuticle (Ikbal and Pavela, 2019; Kimbaris et al., 2010).

Thus, the findings of the model are consistent with the results on bioactivity of EOs and the literature, highlighting the important contribution of trans-cinnamaldehyde, menthol + menthone, estragole and carvone in the high repellency of Chinese cinnamon, peppermint, basil, dill and spearmint oils, respectively, but also the attractiveness of limonene and linalool found in many EOs. These findings act as a first step towards a broad exploration of the effects of plant VOCs on *A. pisum*, which are very poorly known so far, and still require further investigations involving repellency bioassays using pure EO compounds as well as various doses and duration of exposure with biological monitoring. Moreover, particular attention should be given to the mode of application of EOs as biorepellent against the pea aphid due to the high volatility and limited persistence of VOCs under field conditions (rapid conversion and degradation by light, oxygen, humidity and temperature) (Devrnja et al., 2022; Menossi et al., 2021). This problem is enhanced by the lipophilic properties of most EO compounds resulting in low aqueous solubility unsuitable for spray application (Devrnja et al., 2022; Menossi et al., 2021). In this sense, research is heading

towards the development of innovative formulations based on inorganic nanomaterials, lipids and polymers for EO encapsulation that provide a controlled release of VOCs with improved durability and efficiency (Devrnja et al., 2022; Menossi et al., 2021).

3.3. Economic analysis

After identifying the most promising EOs from a biological and chemical standpoint, we considered the commercial development of these EOs as biorepellents against *A. pisum*. The six most repellent EOs were selected, corresponding to class V Chinese cinnamon and dill as well as class IV peppermint, spearmint, anise, and basil. Their repellency, with their availability (number of producing countries) and price (average in France as an example) in conventional or organic quality were cross-referenced in a radar chart (Fig. 6).

The economic analysis of the six EOs revealed that peppermint oil is one of the cheapest, and the best available for a high RI similar than dill and spearmint oils and superior to anise and basil oils. Moreover, according to former literature review, peppermint oil could be effective on attacks of multiple other aphid species than *A. pisum* on the *Fabaceae* crops. Thus, the peppermint EO could be one of the best candidates for the commercial development of biorepellents against the pea aphid *A. pisum* on *Fabaceae* crops, at least on the French market as part of the Ecophyto II+ plan (Le Gouvernement, 2015). However, peppermint oil can be considered as such provided that the EOs produced have a stable content of menthol and menthone as major compounds and that further research has demonstrated its effectiveness in the field.

Finally, as a suggestion, given that research is heading towards EO-loaded biopolymer materials for controlled release of VOCs with less environmental impact (Menossi et al., 2021), peppermint oil could be included in insect nets covering crops, thus combining a chemical and mechanical barrier against aphids and preventing them from reproducing on crops through the meshes of the nets, which often occurs. Thus, taking as an example the anti-insect net FILBIO[7] (Lonobio, Saint-Chef, France) based on polylactic acid (PLA) at 1.46 ex-tax €/m^2, loading the biopolymer with 1% w/w peppermint EO would represent 1.9% of the net price with conventional oil (76.30 ex-tax €/l) and 3.2% with organic oil (127.89 ex-tax €/l).

4. Conclusions

This work addressed the effects of a large selection of plant EOs and their chemical compounds on *A. pisum*, which have been poorly investigated until now. An extensive choice bioassay screening of forty EOs against the pea aphid revealed the high repellency of Chinese cinnamon, peppermint, anise, basil, spearmint and dill oils in controlled conditions. Analysis of their chemical composition showed that their respective high content of the oxygenated VOCs trans-cinnamaldehyde, trans-anethole, menthol + menthone, estragole and carvone could be responsible for their high repellency. An additional economic analysis pointed out that, among the most repellent EOs, peppermint oil has the best availability and the lowest prices in France, as an example where the development of EO-based biorepellents could be part of the Ecophyto II+ plan. Moreover, according to the literature, peppermint oil could be effective against numerous other aphid species on *Fabaceae* crops. Therefore, peppermint EO with menthol and menthone as major compounds appears to be one of the most promising candidates as biorepellent against the pea aphid *A. pisum*.

To address these hypotheses, future work should explore the effect of pure EO compounds as well as additional EO doses and exposure times to more advanced stages of the aphid with interaction with the plant.

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[7] Filet anti insectes FILBIO compostable à mailles fines – Lonobio, (n.d.). https://www.lonobio.fr/produit/filet-anti-insectes-filbio-compostable-a-mailles-fines/ (accessed November 17, 2022).
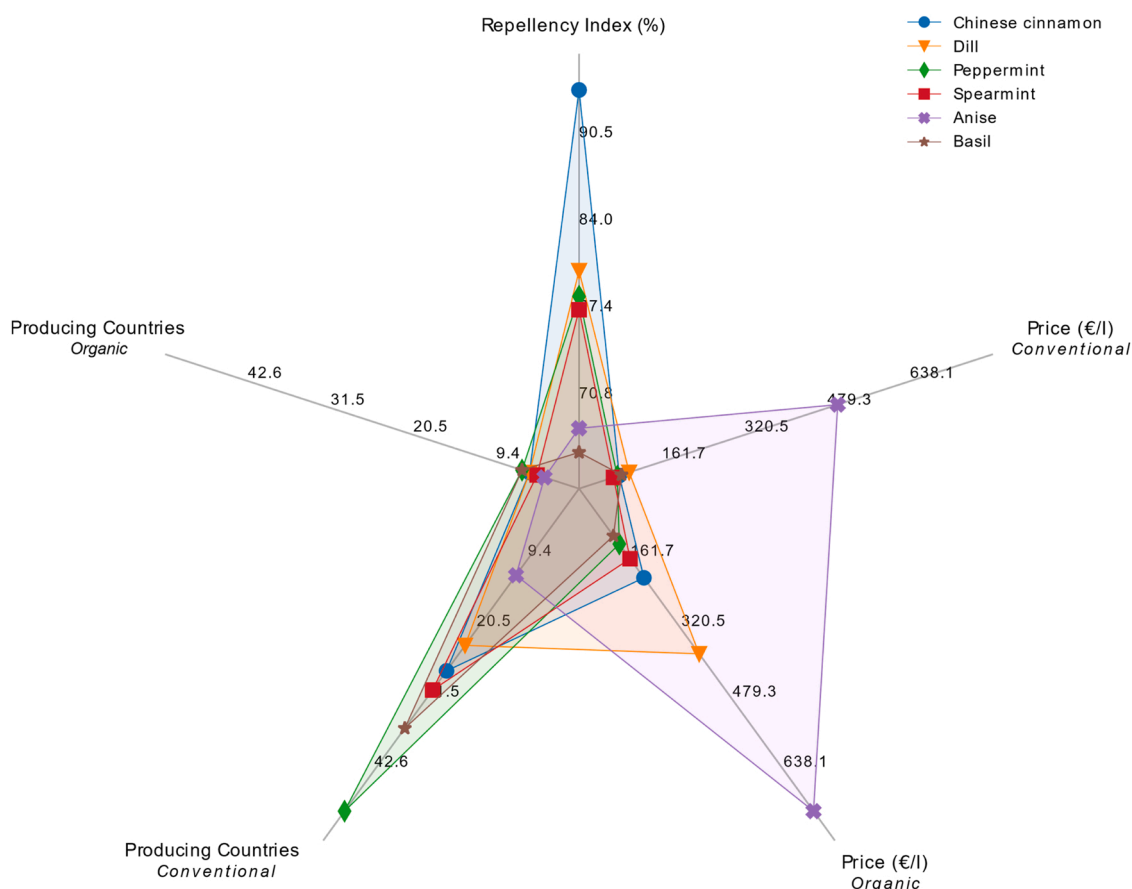
Fig. 6. Economic analysis to consider the commercial development as biorepellents of the six most repellent essential oils (EOs) against the pea aphid *A. pisum*, corresponding to classes V and IV. Their repellency has been cross-referenced with their availability (number of producing countries) and price in conventional or organic quality.

Bioassays with plant interaction could be performed using no-choice and choice tests evaluating aphids feeding behavior on plants. Moreover, in order to consider peppermint oil as a sustainable biorepellent, its phytotoxicity on *Fabaceae* crops and its potential side effects on natural enemies of pea aphid should also be investigated, as existing studies are very limited with rather variable results (Sayed et al., 2022; Kimbaris et al., 2010; Remén, 2005). Finally, the repellency of peppermint oil should be further evaluated in the field for possible use in insect nets, thus combining a chemical and mechanical barrier against aphids with a polymeric material for controlled release of VOCs.

CRediT authorship contribution statement

V. Lacotte: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, **M. Rey:** Methodology, Validation, Investigation, Data curation, Writing – original draft, Writing – review and editing. **S. Peignier:** Conceptualization, Methodology, Software, Formal analysis, Data curation, Writing – original draft, Visualization, **P.-E. Mercier:** Methodology, Validation, Investigation, Data curation, Writing – original draft, Writing – review & editing, **I. Rahioui:** Conceptualization, Methodology, Investigation, **C. Sivignon:** Conceptualization, Methodology, Investigation, **L. Razy:** Conceptualization, Methodology. **S. Benhamou:** Writing – original draft, Writing – review & editing, Visualization, **S. Livi:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition, **P. da Silva:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Declaration of Competing Interest

All authors declare no competing interests, either financial or non-financial.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2023.116610.

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