Acricidal and Insect Antifeedant Effects of Essential Oils From Selected Aromatic Plants and Their Main Components

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This work has demonstrated the ixodicidal and insect antifeedant effects of essential oils from 14 experimentally cultivated aromatic plants. The strong ixodicidal and antifeedant oils corresponded to Thymus zygis, Thymus vulgaris, Satureja montana, Oreganum virens, and Mentha suaveolens. The moderately active oils were from Lavandula angustifolia, Mentha piperita, Mentha spicata, Artemisa herba-alba, and Rosmarinus officinalis. The most effective larvicidal and antifeedant compounds were piperitenone oxide, carvacrol, piperitenone, and thymol, explaining the effects of the most active essential oils. The rest of the tested compounds were not ixodicidal or antifeedant. Therefore, the activity of moderately active oils cannot be explained by their main components (linalyl acetate, linalool, menthone, menthol, limonene, camphor, 1,8-cineole, p-cymene, α-pinene, and carvone), suggesting synergistic effects.

CONCLUSION

This study has demonstrated the potential of 14 experimentally cultivated aromatic plants as new botanical pesticides. The strong ixodicidal and antifeedant oils corresponded to Thymus zygis, Thymus vulgaris, Satureja montana, Oreganum virens, and Mentha suaveolens. The moderately active oils were from Lavandula angustifolia, Mentha piperita, Mentha spicata, Artemisa herba-alba, and Rosmarinus officinalis. The most effective larvicidal and antifeedant compounds were piperitenone oxide, carvacrol, piperitenone, and thymol, explaining the effects of the most active essential oils. The rest of the tested compounds were not ixodicidal or antifeedant. Therefore, the activity of moderately active oils cannot be explained by their main components (linalyl acetate, linalool, menthone, menthol, limonene, camphor, 1,8-cineole, p-cymene, α-pinene, and carvone), suggesting synergistic effects.

Keywords: aromatic plant, essential oil, ixodicidal, antifeedant, Hyalomma lusitanicum, Spodoptera littoralis, Myzus persicae, Rhopas loosiphum padi

INTRODUCTION

Food safety and environmental concerns related to the use of pesticides have resulted in more restricted regulatory frameworks worldwide, reducing the number of commercial products available for crop protection and other pest management sectors including the control of vectors of human and livestock diseases. Therefore, new safer and effective insecticides are needed. Botanical pesticides are emerging as a solution to meet part of the demand (Isman, 2020a). Essential oils (EOs) that are composed of volatile secondary metabolites, mostly terpenes (Bakkali et al., 2008), are among the most important extracts acting as botanical insecticides (Regnault-Roger et al., 2012; Pavela and Benelli, 2016), and some are being commercialized as commercial pesticide ingredients (Isman, 2020b).
Arthropods, including economically important disease vectors and insect pests, are an important target of the biological effects of EOs (Ntalli et al., 2019; Isman, 2020a,b). Tick-borne diseases are a serious health and economic problem, responsible for over 100,000 cases of human diseases worldwide (de la Fuente et al., 2008) and billions of dollars in losses to the livestock industry (Lotfi and Karima, 2020). Additionally, ticks are in expansion due to climate change (Abbas et al., 2018). For example, *Hyalomma* ticks, vectors of the Crimean-Congo hemorrhagic fever virus, have spread from their original distribution (African and Mediterranean environments) to other European countries, becoming an increasing public health concern (Chitimia-Dobler et al., 2019; Hansford et al., 2019; Buczek et al., 2020; Grandi et al., 2020). For many years, tick control has been carried out with synthetic acaricides, leading to the appearance of resistance (reviewed by Abbas et al., 2014) and being harmful to the environment. Therefore, new effective and safer tick control agents are needed. In this context, EOs have been reported as being toxic and/or repellent to ticks (Benelli et al., 2016, 2017a; Benelli and Pavela, 2018; Salman et al., 2020).

Crop yield damages caused by pest infestations and pesticide use are significant (Oerke, 2006; Gregory et al., 2009) and increasing with global warming. Adaptation measures to increased pest damage related to global warming may involve greater use of pesticides with detrimental effects on health, environmental damage, and increased pesticide resistance (Deutsch et al., 2018). Some important crop pests include the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisdulav) (Lepidoptera: Noctuidae), a highly polyphagous insect labeled as an A2 quarantine pest by the OEPP/EPPO (2015) due to its host range (Alford, 2007) and distribution (Centre for Agricultural Bioscience International, 2020a). The green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae), is the most economically important aphid crop pest worldwide (van Emden and Harrington, 2017) due to its distribution (Centre for Agricultural Bioscience International, 2020b), host range (Blackman and Eastop, 2000), mechanisms of plant damage, life cycle, and its ability to evolve resistance to insecticides (Bass et al., 2014). The bird cherry-oat aphid, *Rhopalosiphum padi* L., is a global pest of cereals (van Emden and Harrington, 2017) and a vector of yellow dwarf viruses that cause significant crop losses in cereals (Finlay and Luck, 2011). Many EOs are good insecticidal candidates because of their direct effects, biodegradability, and their low level of toxicity to mammals (Isman, 2020a,b).

The commercial production of a botanical insecticide depends on the sustainable production of plant biomass for extraction. Therefore, the domestication and cultivation of aromatic and medicinal plants (AMPs) for the production of EOs contributes to species conservation and provides sustainability of the production and lower variations in active ingredients. For example, a selected chemotype of wormwood, *Artemisia absinthium* (Asteraceae), that lacks the toxic terpene β-thujone but produces other novel terpenoids that are toxic and antifeedant to a range of pest insects has been domesticated for cultivation and registered as a new plant variety (Gonzalez-Coloma et al., 2017).

As part of an ongoing project on the domestication and valorization of selected AMPs, plant species belonging to the genera *Artemisia*, *Hyssopus*, *Lavandula*, *Mentha*, *Origanum*, *Rosmarinus*, *Satureja*, *Tanacetum*, and *Thymus* have been experimentally cultivated at a small scale. These genera include species traditionally used in medicinal, food, and flavor applications due to their contents in bioactive EOs (Fathiazad and Hamedeyazdan, 2011; Chishiti et al., 2013; Kumar and Tyagi, 2013; Tepe and Gilkiz, 2016; Aprotosoaie et al., 2017; Singh and Pandey, 2018; Borges et al., 2019; Li et al., 2019; Isman, 2020a,b).

In this work, essential oils from selected species of aromatic and medicinal plants cultivated experimentally (Table 1) have been evaluated against arthropods of importance in public health and animal and crop production: the tick (*Hyalomma lusitanicum*) and three insect pests (*S. littoralis*, *M. persicae*, and *R. padii*). *Thymus vulgaris* has been included in this study as a reference to compare the rest of the selected species because it is one of the most important aromatic plants grown worldwide (Southern and Central Europe, Southeast Asia, North America, and Africa), and it is an ingredient of botanical insecticides because of its thymol content (Pavela, 2016). Additionally, the composition of the most active EOs has been analyzed and the oxicidal and insecticidal activities of their main components (Figure 1) tested.

### MATERIALS AND METHODS

#### Plant Material

Fourteen plant species belonging to the families Asteraceae and Lamiaeae (Table 1) were selected for the study. The plants

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**TABLE 1 | List of the plant species used and their origin (experimental field locations in Aragón, Spain, and UTM coordinates).**

| Plant species          | Origin                                                                 |
|------------------------|------------------------------------------------------------------------|
| *Artemisia dracunculus* L. | Ejea de los Caballeros (42°7′45″ N, 1°8′15″ W)                        |
| *Artemisia herba-alba* Asso. | Villafábrica (41°34′28″ N, 0°39′01″ W)                                   |
| *Hyssopus officinalis* L.    | Ejea de los Caballeros (42°7′45″ N, 1°8′15″ W)                        |
| *Lavandula angustifolia* L. | Ejea de los Caballeros (42°7′45″ N, 1°8′15″ W)                        |
| *Mentha piperita* L.          | La Alfranca (41°36′22″ N, 0°45′22″ E)                                  |
| *Mentha spicata* L.            | Ejea de los Caballeros (42°7′45″ N, 1°8′15″ W)                        |
| *Origanum vulgare subsp.*viërens | Fabara (41°10′ N, 0°10′ E)                                            |
| *Rosmarinus officinalis* L.   | Villafábrica (41°34′28″ N, 0°39′01″ W)                                  |
| *Satureja montana* L.         | Ejea de los Caballeros (42°7′45″ N, 1°8′15″ W)                        |
| *Tanacetum vulgare* L.        | Ejea de los Caballeros (42°7′45″ N, 1°8′15″ W)                        |
| *Thymus mastichina* L.        | Moncayo-Trasobares (41°39′49.43″ N, 1°37′48.11″ W)                    |
| *Thymus vulgaris* L.          | Villarroya (41°27′49″ N, 1°47′01″ W)                                  |
| *Thymus zygis* Loefl. ex L.   | Aguarón (41°20′20″ N, 1°16′11″ W)                                     |
Aerial parts of these plants were collected at the flowering stage. EOs were obtained in the laboratory by Clevenger hydrodistillation (European Pharmacopoeia, 1975).

**Essential Oil Analysis**

The essential oils were analyzed by gas chromatography–mass spectrometry (GC-MS) using a Shimadzu GC-2010 gas chromatograph coupled to a Shimadzu GCMS-QP2010 Ultra mass detector (electron ionization, 70 eV) and equipped with a 30-m × 0.25-mm i.d. capillary column (0.25 µm film thickness) Teknokroma TRB-5 (95%) dimethyl–(5%) diphenylpolisiloxane. The working conditions were as follows: split ratio, 20:1; injector temperature, 300°C; temperature of the transfer line connected to the mass spectrometer, 250°C; initial column temperature, 70°C; then heated to 290°C at 6°C/min. The relative amounts of the individual components were calculated based on the peak area without using a correction factor. Electron ionization mass spectra, retention data, and the calculated linear retention indices (LRIs) were used to assess the identity of the compounds by comparing them with those of standards or those found in the Wiley 229 Mass Spectral Database.

**Ixicidal Activity**

*Hyalomma lusitanicum* engorged females were collected from red deer in Ciudad Real (Central Spain) and maintained under laboratory conditions [22–24°C and 80% relative humidity (RH)] until oviposition and egg hatching.

Tick bioassays were performed according to Ruiz-Vásquez et al. (2017). Briefly, 50 µl of the test solution was added to 25 mg of powdered cellulose at different concentrations (initial concentration of 40 or 20 µg/mg for EOs or pure compounds, respectively) and the solvent was evaporated. The ticks and cellulose were then placed in laboratory glass tubes and carefully mixed by rotating the glass several times to ensure full tick–cellulose contact. After mixing, the tubes were kept under laboratory conditions for 24 h. For each test, three replicates with 20 active older than 6 weeks larvae. To validate the tests, three replicates of negative (cellulose, 25 mg) and positive (thymol, 20 µg/mg) controls were also used.

Ticks were considered dead when they could not move from one place to another. Dead ticks were counted after 24 h of contact with the treated cellulose at the laboratory conditions described using a binocular magnifying glass. The larvicidal activity data are presented as percent mortality corrected according to Schneider–Orelli’s formula (Püntener, 1981). Effective lethal doses (LC₅₀ and LC₉₀) were calculated by Probit analysis (1:2 serial dilutions to cover a range of activities between 100 and <50% mortality with a minimum of three doses) (STATGRAPHICS Centurion XVI, version 16.1.02).

**Insect Antifeedant Activity**

*S. littoralis* with two sixth-instar larvae (>24 h old) were allowed to feed in a growth chamber (until 75% larval consumption of leaf disks). Feeding/settling inhibition (%FI or %SI) was calculated as %FI/SI = [1 – (T/C) × 100], where

\[ T = \text{disk area} \]
\[ C = \text{control disk area} \]
TABLE 2 | Larvicidal effects of the selected essential oils on Hyalomma lusitanicum.

| Essential oil                        | % Mortalitya (40 µg/mg) | LD50 (CL)b | LD90 (CL)b |
|--------------------------------------|--------------------------|------------|------------|
| Artemisia dracunculus                | 30.20 ± 11.52            | >40        | >40        |
| Artemisia herba-alba                  | 100                      | 20–40      | 20–40      |
| Hyssopos officinalis                 | 0                        | >40        | >40        |
| Lavandula angustifolia               | 100                      | 16.06 (14.72–17.18) | 19.71 (18.55–21.18) |
| Mentha piperita                      | 100                      | 22.96 (21.06–26.16) | 30.34 (26.9–37.64) |
| Mentha suaveolens                    | 100                      | 4.54 (4.18–4.92) | 6.12 (5.64–6.92) |
| Mentha spicata                       | 100                      | 23.58 (21.48–26.14) | 33.86 (30.58–38.84) |
| Origanum vulgare subsp. virens       | 100                      | 6.38 (5.82–7.00) | 8.96 (8.18–10.10) |
| Rosmarinus officinalis               | 100                      | ~10        | ~12        |
| Satureja montana                     | 100                      | 4.68 (4.14–5.24) | 8.33 (7.54–9.38) |
| Tanacetum vulgare                    | 23.37 ± 6.74             | >40        | >40        |
| Thymus masticina                     | 47.69 ± 20.50            | >40        | >40        |
| Thymus vulgaris                      | 100                      | 5.52 (4.42–6.38) | 9.52 (8.46–11.36) |
| Thymus zygis                         | 100                      | 2.44 (2.18–2.74) | 3.88 (3.48–4.48) |

Values (in percent) are the means of three replicates corrected according to Schneider–Orelli’s formula (Püntener, 1981).

Lethal doses (upper-lower 95% confidence limits) calculated to give 50% (LD50) or 90% (LD90) mortality by Probit analysis.

T and C represent feeding/settling on the treated and control leaf disks, respectively. The antifeedant effects (%FI/SI) were analyzed for significance by the non-parametric Wilcoxon paired signed-rank test comparing the consumption/settling between the treatment and control leaf disks. Extracts and compounds with an SI > 70% were further tested in a dose–response experiment (1:2 serial dilutions to cover a range of activities between 100 and < 50% feeding inhibition with a minimum of three doses) to calculate their relative potency (EC50, the effective dose to give a 50% settling reduction) from the linear regression analysis (%FI/SI on Log-dose, STATGRAPHICS Centurion XVI, version 16.1.02).

RESULTS

Ixodidical Effects
Most of the EOs tested (75%) gave significant ixodidical activity against H. lusitanicum larvae (Table 2), which can be grouped into four categories as follows:

1. Strong ixodidical effects (LC50 < 10 µg/mg): Thymus zygis (four doses tested, 100–46% mortality), followed by Mentha suaveolens (five doses tested, 100–50% mortality), Satureja montana (seven doses tested, 100–18% mortality), T. vulgaris (four doses tested, 100–5% mortality), and Origanum vulgare subsp. virens (six doses tested, 100–10% mortality).
2. Moderate ixodidical effects (LC50 < 16–28 µg/mg): Mentha piperita (three doses, 100–2% mortality), Mentha spicata (three doses, 100–9% mortality), Lavandula angustifolia (two doses, 92–2% mortality), and Rosmarinus officinalis (two doses, 100–20% mortality).
3. Moderate–low ixodidical effects (LC50 < 20–40 µg/mg): Artemisia herba-alba, only toxic at the highest dose tested (40 µg/mg, 100% mortality).
4. No ixodidical effects (LC50 > 40 µg/mg): Artemisia dracunculus, Hyssopus officinalis, Tanacetum vulgare, and Thymus masticina.

Antifeedant Effects
Table 3 shows the insect antifeedant effects of the tested EOs. Overall, the herbivorous insects were less affected by these EOs than the tick (37 and 31% EOs effective against S. litoralis and aphids, respectively).

Spodoptera litoralis feeding was strongly affected by S. montana (four doses, %FI = 90–5, EC50 = 39 µg/cm2), followed by M. piperita, M. spicata, T. vulgaris, T. zygis, and T. vulgaris (%FI = 70–80).

Mentha persicae and R. padi were strongly affected by T. vulgaris (four doses, %SI = 81–10 and 84–7, EC50 = 29 and 49 µg/cm2, respectively) and S. montana (four doses, %SI = 90–5, EC50 = 29 µg/cm2). M. suaveolens (three doses, %SI = 92–20), O. vulgare subsp. virens (three doses, %SI = 78–10), and T. zygis (three doses, %SI = 89–15) showed moderate effects on M. persicae (EC50 = 35, 34, and 45 µg/cm2, respectively). T. zygis, T. vulgaris, and O. vulgare subsp. virens had low effects on R. padi (%SI = 65–70).

Plant Species Ranking
Considering the ixodidical and antifeedant effects of the tested EOs, the plants have been ranked in relation to T. vulgaris (Table 4) for their potential as botanical pesticide ingredients. The ranking index has been established as [T. vulgaris EC50 value/ranked species EC50 value] for each test with significant effects (see Tables 2, 3).

Overall, considering the sum of all the indices, S. montana and T. zygis ranked over T. vulgaris (value > 4). However, T. zygis, S. montana, and M. suaveolens ranked over T. vulgaris as...
TABLE 3 | Insect antifeedant effects of the selected essential oils.

| Essential oil                  | Spodoptera littoralis | Myzus persicae | Rhopalosiphum padi |
|--------------------------------|-----------------------|----------------|-------------------|
|                                | %FIa                  | %SIb           | EC50 (CL)b        |
| Artemisia dracunculus          | 53.4 ± 11             | 21.8 ± 6       | 42.9 ± 7          |
|                                | ~100                  | >100           | >100              |
| Artemisia herba-alba           | 30.2 ± 10             | 59.4 ± 7       | 31.5 ± 7          |
|                                | >100                  | >100           | >100              |
| Hyssopus officinalis           | 40.1 ± 3              | 41.2 ± 8       | 26.6 ± 7          |
|                                | >100                  | >100           | >100              |
| Lavandula angustifolia         | 54.8 ± 11             | 31.0 ± 8       | 46.5 ± 6          |
|                                | ~100                  | >100           | >100              |
| Mentha piperita                | 74.6 ± 8              | 36.5 ± 10      | 33.2 ± 8          |
|                                | >70                   | >100           | >100              |
| Mentha spicata                 | 72.84 ± 12            | 56.7 ± 8       | 17.8 ± 5          |
|                                | >70                   | >100           | >100              |
| Mentha suaveolens              | 71.1 ± 14             | 92.1 ± 3*      | 48.5 ± 7          |
|                                | >70                   | >100           | >100              |
| Origanum vulgare subsp. virens | 37.7 ± 11             | 78.0 ± 7       | 67.2 ± 8*         |
|                                | >100                  | 33.7 (23–50)   | >70               |
| Rosmarinus officinalis         | 35.5 ± 11             | 51.4 ± 6       | 19.9 ± 5          |
|                                | >100                  | ~100           | >100              |
| Satureja montana               | 94.3 ± 1              | 93.5 ± 2*      | 90.1 ± 3*         |
| (15–63)                        | 39.5 ± 2             | 28.9 ± 22       | 29.2 (20–38)      |
| Tanacetum vulgare              | 68.2 ± 10             | 51.8 ± 7       | 68.1 ± 6*         |
|                                | >70                   | ~100           | >70               |
| Thymus mastichina              | 38.7 ± 10             | 33.5 ± 9       | 8.7 ± 5           |
|                                | >100                  | ~100           | >100              |
| Thymus vulgaris                | 74.9 ± 12             | 80.7 ± 6*      | 83.9 ± 5*         |
|                                | >70                   | 29.0 (10–35)   | 49.0 (40–60)      |
| Thymus zygis                   | 72.3 ± 15             | 89.3 ± 5*      | 70.2 ± 8*         |
|                                | >70                   | 45.0 (40–50)   | >70               |

*Percent feeding (FI) inhibition at a dose of 100 µg/cm². Values are the means of five to seven replicates per dose. Values with asterisk are significantly different according to Wilcoxon signed rank test (P < 0.05).

aPercent setting (SI) inhibition at a dose of 100 µg/cm². Values are the means of 20 replicates per dose.

b EC50 (95% lower-upper confidence limits), concentration needed to produce 50% feeding/settling inhibition.

Ixodicidal and Antifeedant Effects of EOs’ Main Components

Table 6 shows the ixodicidal effects of the selected individual components. Piperitenone oxide was the strongest acaricidal compound (LD50−90 = 0.9–1.1 µg/mg), followed by carvacrol (LD50−90 = 1.4–1.7 µg/mg), piperitenone (LD50−90 = 1.8–2.2 µg/mg), and thymol (LD50−90 = 2.9–6.2 µg/mg).

The antifeedant effects of the individual EO components are shown in Table 7. Piperitenone was the most effective antifeedant against S. littoralis (EC50 = 1.4 µg/cm²), followed by piperitenone oxide (EC50 = 5 µg/cm²), thymol (EC50 = 21 µg/cm²), and α-pinene with moderate-low effects (EC50 = ~37 µg/cm²). M. persicae strongly responded to thymol (EC50 = 7.6 µg/cm²) and piperitenone oxide (EC50 = 8.6 µg/cm²), followed by carvacrol (EC50 = 15 µg/cm²) and menthone (EC50 = ~34 µg/cm²). R. padi was the least sensitive insect species and responded to carvacrol (EC50 = 15 µg/cm²), thymol (EC50 = 19 µg/cm²), and menthol oxide (EC50 = ~25 µg/cm²).

EXPERIMENTAL

This work has demonstrated the ixodicidal and insect antifeedant effects of EOs from experimentally cultivated AMPs. Furthermore, more EOs were ixodicidals than insect antifeedants, probably because of their different feeding ecologies (blood sucking vs. herbivores). Ticks are obligate hematophagous ectoparasites (Basu and Charles, 2017) and therefore have not evolved adaptations to plant secondary metabolites. On the other hand, insect herbivores have coevolved with plants and their chemical defenses/secondary metabolites (Maron et al., 2019). These differences in feeding adaptations could explain the selective toxicity of EOs toward the ticks observed here.

The EOs grouped as strong ixodicidal agents corresponded to T. zygis, T. vulgaris, S. montana, M. suaveolens, and Origanum virens. Similarly, the EOs grouped as strong antifeedants corresponded to S. montana, T. zygis, and T. vulgaris, followed by O. virens and M. suaveolens.

Thymus vulgaris EO, an ingredient of botanical pesticides (Pavela, 2016), has been included in this work as a reference for further species selection. In this work, the EO from T. vulgaris (thymol/p-cymene) was the third most ixodicidal and the second most antifeedant against the insect species tested. The most common T. vulgaris chemotypes are thymol/carvacrol (György et al., 2020), which have reported ixodicidal effects including repellency against nymphs of Ixodes ricinus and adults of Dermacentor reticulatus (Stefanidesová et al., 2017; Goode et al., 2018), but not on its larvicidal effects against H. lusitanicum. EO from T. vulgaris has also been described as being insecticidal against several insect species, including S. littoralis, M. persicae (toxicity; Pavela, 2012; Ikbal and Pavela, 2019), and R. padi (antifeedant; Grul’ová et al., 2017). The EO from T. zygis (thymol) was the most effective ixodicidal agent tested here, with insect antifeedant effects similar to T. vulgaris. Previous
reports showed that T. zygis EO (rich in thymol) was ovicidal, larvicial, antifeedant, and repellent against the insect Plutella xylostella (Sangha et al., 2017), but this is the first report on its ixodical activity. T. zygis is distributed in the Iberian Peninsula and north of Africa (Morales Valverde, 1997), the thymol chemotype being of interest (Pérez-Sánchez et al., 2008). Therefore, the high content of thymol (75%) and the effects on ticks of the EO from the T. zygis line tested here support further agronomic development.

The EO from S. montana (carvacrol) was the most effective insect antifeedant and the second most effective ixodidal agent tested in this study. The essential oil of S. montana is characterized by carvacrol, thymol, p-cymene, and linalool (Velasco and Perez-Alonso, 1983; Silva et al., 2009; Dunkic et al., 2012). S. montana EO has reported repellence to Frankliniella occidentalis (Picard et al., 2012), is toxic against Leptinotarsa decemlineata larvae and adults (Usamaz-Bozhuyuk and Kordali, 2018), larvicial against Culex quinquefasciatus (Benelli et al., 2020), and toxic to Drosophila suzukii adults (Park et al., 2016). The population of S. montana used in this work, rich in carvacrol, has already been included in an agronomic development program for the production of biopesticides (Navarro-Rocha et al., 2020). However, this is the first report on the ixodical activity of this EO.

The M. suaveolens population selected for this work was rich in piperitenone oxide/piperitenone. This EO was the second most effective ixodidal extract tested here (more effective than T. vulgaris), along with S. montana, and showed stronger antifeedant effects against M. persicae than T. vulgaris. M. suaveolens is native of Africa, temperate Asia, and Europe (Abbaszadeh et al., 2009). There are three chemotypes described for M. suaveolens: pulegone, piperitenone oxide, and piperitenone oxide/piperitone oxide (Oumzil et al., 2002; Božović et al., 2015). Previously, M. suaveolens EOs (pulegone and menthone) showed ovicidal and larvicidal effects against the tick Hyalomma aegyptium (Laghzaoui et al., 2019). This species’ EOs also have reported insecticidal

### Table 4: Rank index [calculated as EC50 of Thymus vulgaris essential oil (EO)/EC50 of ranked species’ EO] of the bioactive EO-producing plant species tested for further selection.

| Essential oil       | Hyalomma lusitanicum | Spodoptera littoralis | Myzus persicae | Rhopaslosiphum padi | Total index |
|---------------------|-----------------------|-----------------------|----------------|---------------------|-------------|
| Thymus vulgaris     | 1                     | 1                     | 1              | 1                   | 4           |
| Satureja montana    | 1.17                  | 1.67                  | 1              | 1.24                | 6.27        |
| Thymus zygis        | 2.25                  | 0.96                  | 0.64           | 0.69                | 4.53        |
| Mentha suaveolens   | 1.22                  | 0.94                  | 0.83           | 0.66                | 2.99        |
| Origanum vulgare subsp. virens | 0.86         |                       |                 |                     |             |
| Mentha piperita     | 0.23                  | 1                     |                 |                     | 1.23        |
| Mentha spicata      | 0.23                  | 1                     |                 |                     | 1.23        |
| Rosmarinus officinalis | 0.55                  |                       |                 |                     | 0.55        |
| Lavandula angustifolia | 0.34                  |                       |                 |                     | 0.34        |

### Table 5: Main components of the active essential oils.

| Plant species      | Compound (% abundance)                                                                 |
|--------------------|-----------------------------------------------------------------------------------------|
| Artemisia herba-alba| Camphor (19), 1,8-cineole (12), p-cymene (8), borneol (1)                              |
| Lavandula angustifolia | Linalyl acetate (30), linalool (30), geranyl acetate (7), terpineol (4), c-linalool (3), t-linalool (3), caryophyllene (3), neryl acetate (2) |
| Mentha piperita    | Menthone (41), menthol (31), limonene (13)                                              |
| Mentha spicata     | Carvone (79), 1,8-cineole (12), menthol (2)                                              |
| Mentha suaveolens  | Piperitenone oxide (37), piperitenone (21), limonene (7), D-germacrene (7), t-caryophyllene (6) |
| Origanum vulgare subsp. virens | p-Cymene (30), carvacrol (17), linalool (14), a-terpinene (3), myrcene (2), b-caryophyllene (2) |
| Rosmarinus officinalis | Camphor (29), 1,8-cineole (22), a-pinene (11), endoborneol (8), camphene (8), verbenone (5) |
| Satureja montana   | Carvacrol (78), p-cymene (2), borneol (2), thymoquinone (1), 1-octen-3-ol (1)           |
| Thymus vulgaris    | Thymol (49), p-cymene (29), g-terpinene (7), carvacrol (4)                              |
| Thymus zygis       | Thymol (74), p-cymene (9), g-terpinene (7), carvacrol (4)                              |

### Table 6: Ixodidal activity of the main components (% abundance ≥ 10) of the active essential oils on Hyalomma lusitanicum larvae.

| Compound                  | % Mortalitya (20 µg/mg) | LD50 (CL)b | LD90 (CL)b |
|---------------------------|--------------------------|------------|------------|
| α-Pine (S. montana)       | 0                        | >20        | >20        |
| Limonene (S. montana)     | 6.87 ± 1.84              | >20        | >20        |
| Linalool (S. montana)     | 9.73 ± 5.02              | >20        | >20        |
| Cineole (S. montana)      | 3.70 ± 3.70              | >20        | >20        |
| Camphor (M. suaveolens)   | 15.60 ± 4.73             | >20        | >20        |
| p-Cymene (M. suaveolens)  | 5.70 ± 2.97              | >20        | >20        |
| Carvacrol (M. suaveolens) | 100                      | 1.42 (1.34–1.54) | 1.76 (1.62–1.92) |
| Thymol (M. suaveolens)    | 100                      | 2.94 (2.08–3.54) | 6.16 (5.30–7.84) |
| Piperitenone oxide (M. suaveolens) | 100           | 1.77 (1.63–1.92) | 2.19 (2.03–2.40) |
| Piperitenone oxide (M. suaveolens) | 100           | 0.88 (0.81–0.96) | 1.09 (1.02–1.19) |
| Menthone (M. suaveolens)  | 8.50 ± 4.44              | >20        | >20        |
| Menthol (M. suaveolens)   | 31.4 ± 13.6              | >20        | >20        |
| Carvone (M. suaveolens)   | 5.00 ± 2.67              | >20        | >20        |

aValues (in percent) are the means of three replicates corrected according to Schneider-Orelli's formula (Püntener, 1981).
bLethal dosages (upper-lower 95% confidence limits) calculated to give 50% (LD50) or 90% (LD90) mortality by Probit analysis.
TABLE 7 | Antifeedant activity of the main components (% abundance >10) of the active essential oils on Spodoptera littoralis larvae, Myzus persicae, and Rhopalosiphum padi apterous adults in choice tests.

| Compound       | S. littoralis | M. persicae | R. padi | EC50 (CL) |
|----------------|--------------|-------------|---------|-----------|
| α-Pinene       | 67.3±8.9     | 53.9±10.2   | 34.9±8.1| >50       |
| Limonene       | 44.8±14.5    | 29.3±7.7    | 31.1±5.5| >50       |
| Linalool       | 45.3±7.2     | 27.3±7.6    | 48.4±8.3| >50       |
| 1,8 Cineole    | 36.0±8.7     | 56.0±8.5    | 21.9±7.2| >50       |
| Camphor        | 22.6±6.0     | 37.6±7.0    | 38.5±7.6| >50       |
| p-Cymene       | 8.61±6.09    | 20.2±6.50   | 35.0±7.5| >50       |
| Menthone       | 55.8±11.8    | 86.4±3.2*   | 90.6±5.3*| ~50       |
| Thymol         | 52.4±10.1    | 81.8±7.7*   | 92.1±2.6*| ~50       |
| Piperitenone    | 91.8±4.9*    | 56.2±2.4    | nt      | ~1.5 (0.2–9.9) |
| Piperitenone oxide | 90.1±3.7* | 91.1±5.3*   | 75.0±6.5*| ~5.0 (1.8–13.5) |
| Menthol        | 52.9±28.9    | 72.8±9.2*   | 61.6±6.7| >50       |
| Thymol         | 35.6±14.3    | 34.6±8.7    | 45.4±8.7| >50       |
| Carvone        | 52.0±12.7    | 31.0±9.8    | 51.5±8.6| >50       |

*Percent feeding (FI) inhibition at a dose of 100 µg/cm². Values are the means of five to seven replicates per dose. Values with asterisk are significantly different according to Wilcoxon paired rank test (P < 0.05).

*Percent setting (SI) inhibition at a dose of 100 µg/cm². Values are the means of 20 replicates.

*EC50 (95% lower-upper confidence limits), concentration needed to produce 50% feeding/sitting inhibition.

The moderate ixodicidal EOs were from L. angustifolia (linalyl acetate/linalool), M. piperrita (menthone/menthol), M. spicata (carvone/1,8-cineole), R. officinalis (camphor/1,8-cineole/α-pinene), and A. herba-alba (camphor/1,8-cineole/p-cymene). All these EOs were less effective than that of T. vulgaris.

The EO from L. angustifolia tested here (linalyl acetate/linalool) showed moderate ixodicidal effects against H. lusitanicum larvae, lower than the effects of T. vulgaris, without significant insect antifeedant effects. Previous reports have shown interference with the host-seeking behaviors of H. marginatum and D. reticulatus for this species’ EO (Mikolo and Magano, 2007; Stefandidesová et al., 2017) and toxicity to Rhipicephalus (Boophilus) annulatus (Pirali-Kheirabadi and Texeira da Silva, 2010) for this species’ EO. Additionally, a similar EO from the hybrid Lavandula × intermedia (rich in linalyl acetate and linalool) was also toxic to H. lusitanicum larvae and moderately antifeedant to S. littoralis (Ortiz de Elguera-Culebras et al., 2018). L. angustifolia, distributed in the sub-Mediterranean region, has a great economic importance in perfumery, cosmetics, food, pharmaceutical industries, and aromatherapy (Demasi et al., 2018). However, our results do not support its agronomic production as a biopesticide when compared to T. vulgaris, but suggest the valorization of its essential oil production residues (biomass: hydrolate) as a source of biopesticidal ingredients.

M. piperrita (menthone/menthol) was moderately ixodicidal against H. lusitanicum and showed moderate antifeedant effects against S. littoralis. In previous works, M. piperrita EO showed moderate repellency against adults of D. reticularus (Stefandidesová et al., 2017), larvicidal effects against R. microplus (de Souza Chagas et al., 2016), and toxicity against aphids including M. persicae (Ikbal and Pavela, 2019). M. spicata (carvone/1,8-cineole) also had moderate larvicidal effects against H. lusitanicum and moderate-low antifeedant effects on S. littoralis. M. spicata EO has been reported as a moderate...
repellent against adults of *D. reticulatus* (Štefanidesová et al., 2017) and toxic to stored-product pests (Irfan et al., 2009; Kedia et al., 2014; Eriopoulos et al., 2015; Nubia et al., 2016), *L. decemlineata* (Saroukholai et al., 2014), and *S. littoralis* (Pavela, 2005), while a carvone/limonene chemotype of *M. spicata* was not antifeedant or toxic to *S. littoralis*, *M. persicae*, and *R. padi* (Santana et al., 2014). *Mentha* oils are used commercially as biopesticidal ingredients because of their various effects against insects, the most commercialized being the *Mentha* species spearmint (*M. spicata*), peppermint (*M. piperita*), and *M. arvensis* (Singh and Pandey, 2018). Since our results showed lower effects than *T. vulgaris*, we suggest the valorization of their commercial essential oil production residues (biomass: hydrolate) as an additional source of biopesticidal ingredients.

*Rosmarinus officinalis* (camphor/1,8-cineole/α-pinene) showed moderate ixodicial effects in this work. Previous reports have shown a moderate post-ingestive toxicity to *S. littoralis* for a similar *R. officinalis* EO (Santana et al., 2014). *R. officinalis* EOs rich in 1,8-cineole were toxic to larvae of *Hyalomma scapense* (Djebir et al., 2019) and *I. ricinus* nymphs (Elnhalli et al., 2019), while an EO rich in α-pinene showed low–moderate toxicity against larvae of *R. (B.) microplus* (Martinez-Velazquez et al., 2011). This plant is cultivated worldwide as a food flavoring and preservative due to its antioxidant and antimicrobial potential (Borges et al., 2019). Our results showed lower effects for *R. officinalis* than *T. vulgaris*. However, being a commercial plant available worldwide, we suggest the valorization of its essential oil production residues (biomass: hydrolate) as a source of biopesticidal ingredients.

The chemotype of *A. herba-alba* (camphor/1,8-cineole/p-cymene) tested in this work showed low–moderate larvicidal effects against *H. lusitanicum*. *A. herba-alba* is a medicinal and aromatic shrub that grows wild in arid areas of the Mediterranean Basin, being abundant in the Iberian Peninsula (Mohamed et al., 2010), showing chemical diversity (Salido et al., 2004). An *A. herba-alba* EO rich in piperitone showed repellency against *I. ricinus* nymphs (El-Seedi et al., 2017), and a thujone/camphor chemotype was antifeedant and moderately toxic to *S. littoralis* (Santana et al., 2014). Our results showed lower effects for a camphor/1,8-cineole *A. herba-alba* chemotype than those reported on *T. vulgaris*. Given the chemical diversity of *A. herba-alba* wild populations, we suggest further research on chemotype–bioactivity correlations for this plant species prior to its selection for agronomical development.

Considering the plant species’ rank based on the ixodicial and antifeedant effects of their EOs, we propose the plant populations of *S. montana*, *T. zygis*, and *M. suaveolens* tested here for further agronomical development as biopesticidal ingredients for the control of ticks and insects. These EOs (*S. montana*, *T. zygis*, and *M. suaveolens*) have additional biopesticidal effects such as strong nematicidal action against root-knot nematodes (*Meloidogyne javanica*), with *S. montana* being the most effective (LC$_{50} = 0.041$ μg/μl) (Andrés et al., 2012).

To further understand the effects of the active EOs, their main components (*Figure 1*) were also tested against the selected targets. The most effective larvicidal and antifeedant compounds were piperitenone oxide, carvacrol, piperitenone, and thymol. The activity of piperitenone oxide and piperitenone explained the effects of *M. suaveolens* EO. Thymol explained the effects of the EOs from *T. zygis* and *T. vulgaris*, while carvacrol was responsible for the effects of *S. montana* and *O. vulgar* subsp. *virens*.

These compounds have reported ixodicial and/or insecticidal effects. Piperitenone epoxide and piperitenone showed strong larvicidal and repellent effects against *Aedes albopictus* (Giatropoulos et al., 2018). Piperitenone was antifeedant to *L. decemlineata* and *S. littoralis* (Kimbaris et al., 2017). However, there are no reports on the acaricidal effects of these compounds. Thymol was larvicidal to *H. lusitanicum* (Navarro-Rocha et al., 2018), and carvacrol was toxic to *Rhipicephalus turanicus* (Coskun et al., 2008) and moderately toxic to *Hyalomma marginatum* adults (Cetin et al., 2010). These compounds were repellent to *Amblyomma americanum* (Carroll et al., 2017) and showed strong toxicity against *I. ricinus* larvae and repellency against *I. ricinus* larvae and *A. americanum* nymphs (Carroll et al., 2017; Tabari et al., 2017). Carvacrol and thymol also have reported behavioral and toxic effects against several insect species, including the ones targeted here. Specifically, thymol was antifeedant to *M. persicae* (Navarro-Rocha et al., 2018). Thymol and carvacrol were antifeedant to *S. littoralis* fourth-instar larvae (Pavela, 2011) and affected the olfactory sensilla of female *S. littoralis* adults (Anderson et al., 1993). Additionally, carvacrol and thymol showed acute toxicity to *S. littoralis* third-instar larvae (Pavela, 2014), and carvacrol was toxic to *M. persicae* (Petrakis et al., 2014).

The rest of the tested compounds were not ixodicial or antifeedant. Therefore, the activity of the moderately active EOs (*L. angustifolia*, *M. piperita*, *M. spicata*, *R. officinalis*, and *A. herba-alba*) cannot be explained by their main components (linalyl acetate, linalool, menthone, menthol, limonene, camphor, 1,8-cineole, p-cymene, α-pinene, and carvone), suggesting synergistic effects. *p*-Cymene was among the most frequent synergists found, interacting with 22 terpenes commonly present in EOs (Pavela et al., 2014). Therefore, synergistic interactions among EO components could explain their ixodicial effects.

**CONCLUSION**

This work has demonstrated the ixodicial and insect antifeedant effects of EOs from experimentally cultivated AMPS. The EOs grouped as strong ixodicial agents corresponded to *T. zygis*, *T. vulgaris*, *S. montana*, *M. suaveolens*, and *O. vulgar* subsp. *virens*. Similarly, the EOs grouped as strong antifeedants corresponded to *S. montana*, *T. zygis*, and *T. vulgaris*, followed by *O. vulgar* subsp. *virens* and *M. suaveolens*. The moderate ixodicial EOs were from *L. angustifolia*, *M. piperita*, *M. spicata*, *A. herba-alba*, and *R. officinalis*.

The most effective larvicidal and antifeedant compounds were piperitenone oxide, carvacrol, piperitenone, and thymol, explaining the effects of *M. suaveolens*, *T. zygis*, *T. vulgaris*, *S. montana*, and *O. vulgar* subsp. *virens* EOs. The rest of the tested compounds were not ixodicial or antifeedant. Therefore, the activity of the moderately active EOs (*L. angustifolia*, *M.
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DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AG-C conceptualized the study. AG-C, AO, MG, JN-R, and FV curated the data. MG, FV, and MA did the formal analysis. AG-C, AO, and FV helped with funding acquisition and resources. MG, JN-R, FV, AO, MA, and AG-C did the investigation. AO, FV, AG-C, and MA helped with the methodology. FV and AG-C wrote the original draft. AG-C, AO, MA, FV, and JN-R did the writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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