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Repellent activity of *Origanum vulgare* and *Artemisia herba-alba* essential oils and their major components against the spotted wing *Drosophila suzukii*

Flavio Polito[1] | Vincenzo De Feo[1] | Roberto Rosamilia[2]* | Paolo Fanti[2] | Donatella Battaglia[2] | Vincenzo Trotta[2]

The widespread use of synthetic pesticides in agricultural contexts has caused various environmental and health issues, including contamination of food, soil and water, as well as the development of resistance in target pests. Biopesticides and plant-derived natural compounds are increasingly promoted as sustainable alternatives due to their reduced toxicity, enhanced biodegradability and compatibility with organic farming. *Drosophila suzukii* is an invasive pest that poses a significant challenge due to its preference for ripe, ready-to-eat fruits, and the limited effectiveness of biological control methods. This study investigates the repellent activity of *Origanum vulgare* and *Artemisia herba-alba* essential oils, as well as their key constituents (carvacrol, camphor, and α-thujone), against *D. suzukii*. The composition of essential oils was determined by GC-MS and their main compounds- α-thujone (9.6%) and camphor (26.02%) in *A. herba-alba*, and carvacrol (67.05%) in *O. vulgare*- exhibited a repellent activity against *D. suzukii*. Repellency index values in the two-choice trap assay were 0.37 ± 0.11 for α-thujone, 0.28 ± 0.07 for camphor, 0.58 ± 0.13 for carvacrol, and -0.57 ± 0.13 for *O. vulgare* EO. In no-choice oviposition bioassays, carvacrol reduced egg-laying by 51% compared to controls, whereas in paired-choice oviposition assays, *O. vulgare* EO stimulated egg-laying. Overall, our results contribute to the development of alternative pest management strategies against *D. suzukii* that align with the principles of integrated pest management and organic farming, thereby reducing dependence on synthetic chemical pesticides.

Keywords: Biopesticides, Essential oils, *Origanum vulgare*, *Artemisia herba-alba*, *Drosophila suzukii*, Repellency, Invasive pest control

INTRODUCTION

Agrochemicals (pesticides and other categories of products related to plant health) are used to increase crop yields by reducing pest infestation. The massive use of conventional chemical pesticides in agriculture in recent decades has led to adverse effects on humans and non-target organisms, and, more generally, to significant long-term changes in the environment. In addition, pesticide residues can contaminate foods, animal feed, soil, and water because many of them have long half-life[1]. Pesticide residues can potentially contaminate many foods, including fruits and vegetables, fruit juices, milk, eggs, wine, and animal feed. Fruit and vegetables are often treated with pesticides at different stages of production, depending on the specific insect pests affecting the crop. Consequently, levels of pesticide residue can vary not only between different types of products, but also between production sites for the same crop. As one of the current priorities of agricultural policy is to reduce the adverse effects of chemicals on human health[2], the use of natural compounds could be considered as a valid alternative due to their relatively lower toxicity and higher biodegradability. The development of biopesticides is very important for crop protection, as they represent an interesting tool in integrated pest management (IPM) and are compatible with organic farming. They can also be used as an alternative to other chemicals that are restricted by legislation or have led to the development of resistance[3]. The use of biopesticides is increasing and accounts for about 10% of the global pesticide market[4]. This is mainly due to the increased return on investment and because they are (or should be) sustainable, environmentally friendly, have low toxicity to non-target organisms, and minimal

[1] Dipartimento di Farmacia, Università of Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano, Italy
[2] Dipartimento di Scienze Agrarie, Forestali, Alimentari ed Ambientali (DAFE), Università degli Studi della Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy

***Corresponding Author** Roberto Rosamilia (roberto.rosamilia27@gmail.com)

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residue management and adverse effects on human health[3,4]. In addition, biopesticides represent a new and interesting scientific tool.

The Asian vinegar fly *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), also called "spotted wing drosophila", is the most problematic invasive pest species damaging the soft-skinned fruit production worldwide[5]. These flies, native to Southeast Asia, have quickly expanded their range to include the Americas, Europe, and recently, Africa. The spotted-wing drosophila is attracted to ripening or ripe fruits and oviposits in them. In addition to the direct damage to the fruit, the wounds caused by the females during deposition facilitate secondary microbial infestation of the fruit[6]. Chemical insecticides continue to be the primary method of controlling *D. suzukii*. Although many insecticides, including pyrethroids, organophosphates, and spinosyns, are effective against *D. suzukii*, others show limited efficacy, posing an important challenge in terms of pest management, particularly in organic farming systems[5]. Pesticides effective against *D. suzukii* have different modes of action, e.g., acetylcholinesterase inhibitors, sodium channel modulators, and ryanodine receptor modulators[7]. However, a major concern with the use of chemical insecticides to control *D. suzukii* is the unwanted residues left on fruits, as this insect attacks ripe fruits, which are usually ready to eat. There is therefore a strong interest in controlling *D. suzukii* using methods other than synthetic pesticides[7].

Adults of *D. suzukii* utilize several cues from ripe host fruits and symbiotic microbes to detect suitable sites for feeding, mating, and oviposition, and volatiles play a central role in mediating these behaviours. Odours from fermentation products (ethanol and acetic acid, but also other compounds) strongly attract *D. suzukii* flies for feeding and mating[8]. For example, *D. suzukii* is strongly attracted by red wine and apple cider vinegar, as they use ubiquitous odours from fermentation products to locate the potential habitat suitable for feeding, mating, and oviposition[9].

A key tool in *D. suzukii* management programs could be the use of natural compounds that repel or attract the flies. Numerous plant species have shown repellent effects on various insect species due to their secondary metabolites, which can result in repellent properties being manifested through various mechanisms[10]. Essential oils (EOs), which are extracted from various plants, are emerging as a natural alternative to synthetic chemicals, due to their insecticidal properties and behavioral effects, which include insect repellency and attraction[5]. In addition, EOs have the advantage of being easy to extract and biodegradable, with no adverse effect on soil or water. For example, EOs extracted from oregano (*Origanum vulgare* L.) have shown remarkable repellent activity against various dangerous species. *O. vulgare* is a perennial plant that belongs to the Lamiaceae family. It is native to temperate regions of Europe and Asia, and is one of the most famous spices used in cooking to flavour dishes, thanks to its strong aroma and pungent flavour. It is widely recognized for its antimicrobial, antioxidant and anti-inflammatory properties, mainly attributed to its rich content of phenolic compounds such as carvacrol and thymol[11]. Its numerous applications in cooking, phytotherapy, and natural food preservation make it one of the most studied and appreciated medicinal herbs globally. Its repellent activity is mainly due to compounds such as thymol, carvacrol, and 1,8-cineole, all of which are typically found in high concentrations in its EOs[12,13].

Similarly, the EO of *Artemisia herba-alba* Asso exhibits strong insect-repellent activity: this effect is primarily due to the presence of thujone, 1,8-cineole, and camphor, compounds known for their neurotoxic and behavioral disruption effects on various insect species[14,15]. *A. herba-alba*, also known as white wormwood or desert wormwood, is a spontaneous aromatic plant of the Asteraceae family, widespread in the arid regions of the Mediterranean and North Africa. Its antidiabetic, antimicrobial, and antiparasitic activities make it useful in numerous traditional medicines[16] . Dominant components found in the EO of this plant are 1,8-cineole, camphor, α-thujone, and β-thujone [17].

The aim of this study was to address the need for alternative, sustainable, environmentally friendly, and low-toxicity botanical repellents for *D. suzukii*[18,19], supporting the development of alternative, eco-compatible pest management strategies. Specifically, we investigated EOs extracted from *O. vulgare* and *A. herba-alba*, along with their major components, carvacrol, camphor, and α-thujone. We hypothesized that these compounds may exert repellent or attractive effects on the flies, and their individual activity could differ from the whole oils. We further hypothesized that the identification of the most active constituents would provide a mechanistic basis for the rational design of botanical-based repellents, thereby supporting their integration into pest management programs, adoption in organic farming systems, and advancement toward commercial application. In contrast to previous studies that have focused on either whole EOs or single pure compounds, our work offers a comparative evaluation of EOs and their major constituents. This highlights potential differences in the activity of whole EOs and their individual metabolites. This dual-level approach enables us to better understand how specific molecules contribute to repellency and attractiveness. This issue has not yet been systematically addressed for *D. suzukii*. Moreover, few studies have examined repellency and oviposition behavior together for this insect pest. Thus, our work provides new insights into how EOs and their key compounds may simultaneously influence different aspects of insect-pest biology. This study contributes to the growing body of research on sustainable alternatives to synthetic insecticides and on the potential use of plant-derived compounds in eco-friendly pest control.

Materials and Methods

Insect rearing

The colony of *D. suzukii* used in this study was obtained from the Fondazione Edmund Mach (Trento, Italy; 46.192° N, 11.136° E). Spotted wing drosophila were reared on a standard diet and no chemicals were used on this strain from the time of collection until the start of the experiment. Flies were reared in the laboratory in a Binder KBF climate chamber at 22±1°C, 75±5% relative humidity (RH) and an 18:6 Light:Dark (L:D) photoperiod. The female flies used in each experiment were sexually mature (3-5 days old) and were starved for approximately 4 h prior to the experiments.

Plant materials

Aerial parts of *A. herba-alba* were collected in flowering stage in September 2023 near the city of Siliana (Bargou region, 36°4′55″N, 9°22′29″E), North West of Tunisia. The identification was made by Dr. I. Amri, Laboratory of Biotechnology and Nuclear Technology, National Center for Nuclear Sciences and Technologies (CNSTN), Sidi Thabet 2020, Tunisia. A voucher specimen (Her172) was stored in the herbarium section of the Institut National de la Recherche en Genie Rural, Eau et Forest, INRGREF, Tunisia.

Aerial parts of *O. vulgare* were collected in July 2023 in flowering stage in S. Arsenio (Salerno, South Italy, 40°28′N 15°29′E) and identified by Prof. V. De Feo, Department of Pharmacy, University of Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano, Italy. A voucher specimen (DFV2023-157) is stored in the herbarium section of the Department of Pharmacy, University of Salerno, Italy.

The plants were air-dried for 15 days and then stored in a dark, humidity-controlled environment until they were ready for use.

Extraction of EOs

Aerial parts of both plants were steam-distilled for 2 h, according to the method reported by the European Pharmacopoeia[20]. The EOs were solubilized in *n*-hexane, filtered over anhydrous sodium sulphate and stored under N_2 at +4°C in the dark until tested and analyzed.

GC-MS analysis

The chemical composition of the EOs was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The operating conditions were the same as those reported in other works by the same authors[21,22]. Most of the components were identified by comparing their Kovats indices (KI) with those of the literature[23] and by a careful analysis of the mass spectra compared to those of the pure compounds available in our laboratory or to those present in the NIST 14 and Wiley 257 mass libraries[24]. The Kovats indices were determined in relation to a homologous series of *n*-alkanes (C10-C35), under the same operating conditions. For some compounds, the identification was confirmed by co-injection with standard samples. Relative concentrations of the components were calculated by peak area normalization. Response factors were not considered.

Chemicals

All pure compounds tested (carvacrol, camphor and α-thujone) and solvents were purchased from Sigma Aldrich (Milan, Italy).

Repellent solutions

Working repellent solutions were prepared using the same protocol for all compounds: 1 µL of each substance was diluted in 10 µL of acetone, and the volume was brought up to 1 mL with distilled water. This concentration was selected as it is commonly employed in behavioral bioassays with natural compounds and represents a practical compromise for potential commercial applications in pest management[25]. For EOs extracted from *A. herba-alba* and *O. vulgare*, concentrations are 0.1% (v/v), while for pure compounds, concentrations are expressed in mM, with values corresponding to 6.04 mM, 6.5 mM, and 6.6 mM for α-thujone, carvacrol, and camphor, respectively. A control solution was prepared by diluting 10 µL of acetone in 1 mL of distilled water.

Behavioral bioassays

The T-maze assay was adapted from previously established methods, with some modifications[26]. Briefly, two 1 mL pipette tips and two 1.5 mL microcentrifuge tubes were cut and combined to form two traps and connected with a 4 cm plastic tube. A piece of filter paper (0.5 cm × 3 cm) was placed against the wall of the cut end of the microcentrifuge tube and fixed with a forceps. A 15 µL diluted compound or control solution was applied to the filter paper. Shortly after the application, 10 mature *D. suzukii* females were introduced into the plastic tube using a pipette tip connected to the tube through a small hole in the centre of the tube. T-maze setups were individually placed horizontally in the climate chamber under controlled environmental conditions at 22±1°C and an 18:6 L:D photoperiod, with the position of the test and control tubes alternating. Before conducting the experiments, the T-maze assay method was validated using apple cider vinegar as the attractant (positive control) and water as the negative control, with five replicates of ten flies each (N = 50 flies).

The two-choice trap assay used in this experiment was adapted from previously established methods[26]. Ten adult females were gently introduced into a plastic cylinder (diameter: 5.5 cm, volume: 150 mL) with a mesh-covered ventilation hole in the screw cap. Two inverted 1.5 mL

microcentrifuge tubes, cut at the base, were placed inside the plastic cylinder. Two pipette tips (1 mL) were cut off at both ends and inserted into the open cut of these inverted microcentrifuge tubes, resembling a funnel. A piece of filter paper was placed against the wall of the cut end of the microcentrifuge tube. Then, 15 µL of EOs (at 0.1% v/v concentration) or pure compounds (α-thujone at 6.04 mM, carvacrol at 6.5 mM, and camphor at 6.6 mM), or the control solution was applied to the filter paper, as for the T-maze assay. As an attractant, 250 µL of a 1:1 (v/v) mixture of apple cider vinegar and distilled water was applied to the inverted cap of each microcentrifuge tube.

Trials were conducted under previously described controlled environmental conditions over a period of 24 h, during which the number of flies entering each trap was counted. Fresh flies and new tubes or plastic cylinders were used for each trial. The T-maze and choice assays were conducted in three separate periods, 7 days apart, resulting in three experimental runs (3 independent replicates). Each run consisted of 5 tubes or plastic cylinders (N = 5 compounds × 3-time intervals × 5 tubes × 10 flies = 750 total flies per assay). Only trials in which at least 6 out of 10 flies entered the control or treated chamber were used for further analysis, sometimes reducing the number of trials per replicate to 4. Before conducting the experiments, the two-choice trap assay was validated using only apple cider vinegar in both traps (positive control), with three replicates of twenty flies each (N = 60).

The Repellency Index (RI) was used to better visualise the repellency data from the T-maze and choice assays[27]. RI was calculated as (C − O) / (C + O), where C is the number of flies in the control trap, and O is the number of flies in the test odorant compound trap.

Oviposition bioassays

Paired choice and no-choice assays were conducted to determine the repellent effects of the tested compounds on *D. suzukii* oviposition, following the methods of Quadrel *et al.*[28]. For these assays, 75 mL plastic cups containing 50 mL of artificial diet were used. A 100 µL of EOs (at 0.1% v/v concentration) or pure compounds (α-thujone at 6.04 mM, carvacrol at 6.5 mM, and camphor at 6.6 mM), or a control solution was applied to the surface of the diet. The treated diet was then allowed to dry under a fume hood for 1 hour before testing. Cups containing treated diets were then placed at the two ends of a larger plastic box (30 × 20 × 15 cm) with a mesh-covered ventilation hole in the screw cap, and maintained in a climate chamber under controlled environmental conditions of 22±1°C and an 18:6 L:D. Each experiment was conducted in two separate periods, 7 days apart, resulting in two independent runs. Each run consisted of 5 plastic boxes. For the oviposition paired choice assay, choice arenas were constructed by placing a diet cup treated with the EO or pure compound on one side of the plastic box, and a control diet cup at the opposite side. The same protocol was used for the no-choice assays, except that both diet cups were treated with the same compound. Additionally, a control group containing two untreated diet cups was included, resulting in five experimental groups. Ten mated *D. suzukii* females were introduced into each arena. After 24 hours, the flies were counted and removed, and the diet cups were examined under a stereomicroscope to count the number of eggs laid. The mean number of eggs laid per female was calculated by dividing the total number of eggs observed by the number of surviving females.

Statistical analysis

Data from the T-maze and choice assays are presented as means of RI±standard error. For each compound, data were analyzed separately using a Chi-square test, based on the null hypothesis that flies had an equal probability of choosing the control or treated diet. Only flies that made a choice were included in the analysis. Prior to pooling data, the homogeneity of the three replicates for each compound was assessed using a Chi-squared test applied to a 2 x 3 contingency table.

Data from the oviposition paired-choice assay were analyzed using two-tailed Student's *t*-tests to compare the mean number of eggs laid per female on the control versus the treated medium. For no-choice oviposition bioassays, data were analyzed using a linear model (LM), as normality was confirmed via Shapiro-Wilk tests, followed by pairwise comparisons among groups using the Tukey HSD *post hoc* test (α = 0.05). In the LM, the dependent variable was the number of eggs laid by a female, with "treatment" as the main factor (six levels: five compounds and a control) and "replicate" nested within treatment.

Results were considered statistically significant at $p < 0.05$. All data were analysed using the statistical software R version "4.4.1"[29].

Results

Chemical composition of the EOs

The EO of *O. vulgare* showed a yield of 1.8% on dry weight. As shown in Table 1, 18 components were identified in the EO of *O. vulgare*, accounting for 97.43±0.41% of the total. Monoterpenes were the main class, divided into oxygenated (72.12±0.65%) and hydrocarbons (20.55±0.12%). Sesquiterpenes were present in much lower quantities (2.25±0.05%) for hydrocarbons and 1.15±0.13% for oxygenates. The main component was carvacrol (67.11±0.76%), followed by *p*-cymene (18.03±0.41%).

The EO of *A. herba-alba* showed a yield of 0.1% on a dry weight basis. The composition of the EO of *A. herba-alba,* reported in Table 2, has already been studied in a previous work by some of the authors[30]. In summary,

Table 1. Chemical composition of the *O. vulgare* EO

No.	Compound	%	Ki[a]	Ki[a1]	Ki[b]	Ki[b1]	Identification[c]	Chemical class
1	1-Octen-3-ol	0.37±0.04	980	976	1452	1451	1,2	Alcohol
2	β-Myrcene	0.44±0.07	991	992	1161	1166	1,2	Monoterpene hydrocarbon
3	α-Terpinene	0.55±0.07	1012	1017	1178	1183	1,2,3	Monoterpene hydrocarbon
4	*p*-Cymene	18.03±0.41	1023	1021	1270	1279	1,2,3	Aromatic monoterpene hydrocarbon
5	*trans*-β-Ocimene	0.32±0.05	1025	1022	1250	1262	1,2	Monoterpene hydrocarbon
6	γ-Terpinene	1.21±0.11	1056	1060	1245	1253	1,2,3	Monoterpene hydrocarbon
7	*endo*-Borneol	1.25±0.11	1161	1162	1700	1715	1,2,3	Monoterpenoid alcohol
8	Terpinen-4-ol	1.10±0.09	1174	1171	1601	1616	1,2,3	Monoterpenoid alcohol
9	Carvacrol methyl ether	0.58±0.09	1241	1245	1599	1586	1,2	Monoterpenoid aromatic ether
10	*o*-tert-Butyl-o-cresol	0.66±0.08	1281	1278	2260	-	1,2	Alkylated phenol
11	Thymol	1.03±0.11	1289	1292	2164	2172	1,2,3	Monoterpenoid phenol
12	Carvacrol	67.11±0.76	1309	1307	2211	2215	1,2,3	Monoterpenoid phenol
13	β-Caryophyllene	1.07±0.10	1406	1410	1598	1604	1,2	Sesquiterpene hydrocarbon
14	β-Bisabolene	0.66±0.07	1505	1503	1728	1729	1,2	Sesquiterpene hydrocarbon
15	*p*-Cymene-2,5-diol	0.68±0.08	1554	1559	2176	-	1,2	Phenolic diol
16	Caryophyllene oxide	1.15±0.13	1573	1578	1986	1989	1,2	Sesquiterpene oxide
17	γ-Cadinene	0.51±0.08	1511	1514	-	1770	1,2	Sesquiterpene hydrocarbon
18	2-Hydroxy-5-methoxy-acetophenone	0.70±0.076	1618	1616	-	-	1,2	Aromatic ketone
Total		97.43±0.41						
Monoterpene hydrocarbons		20.55±0.12						
Oxygenated monoterpenes		72.12±0.65						
Sesquiterpene hydrocarbons		2.25±0.05						
Oxygenated sesquiterpenoids		1.15±0.13						
Others		1.36±0.28						

[a,b]Kovats retention indices determined relative to a series of *n*-alkanes (C10–C35) on the apolar HP-5 MS (Ki[a]) and the polar HP Innowax capillary columns (Ki[b]), respectively; [a1,b1]Kovats indices from literature; [c]Identification method: 1 = comparison of the Kovats retention indices with published data, 2 =comparison of mass spectra with those listed in the NIST 02 and Wiley 275 libraries and with published data; 3 = co-injection with authentic compounds; The results are the mean of three experiments±SD

the EO contains 67 components (95.49±0.24% of the total) with a predominance of oxygenated monoterpenes (79.27±1.23%) of which camphor (26.22±0.67%), α- and β-thujone (9.85±0.63 and 9.17±0.71%, respectively) were the major components.

Behavioral bioassays

For the T-maze assay, the method was first validated using apple cider vinegar as a positive control and water as a negative control, confirming that *D. suzukii* adult individuals were strongly attracted to vinegar (repellency

Table 2. Chemical composition of the *A. herba-alba* EO

No.	Compound	%	Ki[a]	Ki[a1]	Ki[b]	Ki[b1]	Identification[c]	Chemical class
1	Santolina triene	0.18±0.01	906	908	1036	1043	1,2	Monoterpene hydrocarbon
2	Tricyclene	0.35±0.03	914	917	1012	1008	1,2	Monoterpene hydrocarbon
3	α-Pinene	1.08±0.08	926	929	1025	1027	1,2,3	Monoterpene hydrocarbon
4	Camphene	4.97±0.89	940	936	1068	1092	1,2,3	Monoterpene hydrocarbon
5	Sabinene	0.77±0.14	948	944	1122	1125	1,2	Monoterpene hydrocarbon
6	Dehydro-1,8-cineole	0.06±0.02	981	978	1192	1195	1,2	Monoterpenoid ether
7	α-Phellandrene	0.05±0.02	994	996	1168	1160	1,2,3	Monoterpene hydrocarbon
8	Yomogi alcohol	0.18±0.02	998	999	1395	1403	1,2	Monoterpenoid alcohol
9	α-Terpinene	0.14±0.01	1012	1017	1178	1183	1,2,3	Monoterpene hydrocarbon
10	o-Cymene	0.90±0.08	1017	1021	1310	1291	1,2	Aromatic monoterpene hydrocarbon
11	1,8-Cineole	7.64±0.34	1021	1023	1211	1220	1,2,3	Monoterpenoid ether (oxide)
12	Santolina alcohol	0.25±0.04	1031	1036	-	-	1,2	Monoterpenoid alcohol
13	γ-Terpinene	0.15±0.03	1056	1060	1245	1253	1,2,3	Monoterpene hydrocarbon
14	Terpinolene	1.07±0.09	1062	1067	1282	1291	1,2,3	Monoterpene hydrocarbon
15	*trans*-Sabinene hydrate	0.27±0.02	1063	1060	2092	-	1,2	Monoterpenoid alcohol
16	α-Thujone	9.85±0.63	1095	1099	1423	1451	1,2,3	Monoterpenoid ketone
17	β-Thujone	9.17±0.71	1112	1116	1440	1442	1,2,3	Monoterpenoid ketone
18	Chrysantenone	0.86±0.09	1115	1119	1508	1540	1,2	Monoterpenoid ketone
19	*trans*-*p*-Menth-2-en-1-ol	0.71±0.12	1118	1122	1584	1563	1,2	Monoterpenoid alcohol
20	*cis*-Verbenol	0.07±0.01	1123	1128	1660	1663	1,2	Monoterpenoid alcohol
21	Camphor	26.22±0.67	1136	1139	1515	1531	1,2,3	Monoterpenoid ketone
22	Camphene hydrate	0.24±0.03	1141	1146	1602	-	1,2	Monoterpenoid alcohol
23	Pinocarvone	1.31±0.15	1162	1164	1576	-	1,2	Monoterpenoid ketone
24	Borneol	3.42±0.29	1164	1162	1670	1715	1,2,3	Monoterpenoid alcohol
25	*p*-Mentha-1,5-dien-8-ol	0.18±0.03	1165	1166	1674	1674	1,2	Monoterpenoid alcohol
26	Terpinen-4-ol	0.48±0.05	1174	1171	1601	1616	1,2,3	Monoterpenoid alcohol
27	Myrtenal	1.25±0.35	1194	1197	1632	1648	1,2	Monoterpenoid aldehyde
28	Myrtenol	0.27±0.02	1204	1209	1790	1807	1,2	Monoterpenoid alcohol
29	Verbenone	0.67±0.08	1207	1212	1726	1733	1,2	Monoterpenoid ketone
30	*trans*-Piperitol	0.26±0.05	1208	1211	1710	-	1,2	Monoterpenoid alcohol
31	*trans*-Carveol	0.14±0.01	1215	1217	1720	1832	1,2	Monoterpenoid alcohol
32	(E)-Ocimenone	0.17±0.01	1230	1230	-	-	1,2	Monoterpenoid ketone
33	Piperitenone	4.59±0.61	1253	1252	1909	1918	1,2	Sesquiterpene hydrocarbon
34	Car-3-en-2-one	1.39±0.23	1257	1254	-	-	1,2	Monoterpenoid ketone
35	Piperitone	0.50±0.07	1263	1268	1730	1748	1,2	Monoterpenoid ketone

Table 2 ***cont***.

No.	Compound	%	Ki[a]	Ki[a1]	Ki[b]	Ki[b1]	Identification[c]	Chemical class
36	*cis*-Verbenyl acetate	0.82±0.17	1282	1280	-	-	1,2	Monoterpenoid ester
37	*p*-Mentha-1,8-dien-3-one	1.55±0.22	1283	1285	-	-	1,2	Monoterpenoid ketone
38	Bornyl acetate	0.81±0.04	1286	1287	1579	1591	1,2	Monoterpenoid ester
39	*trans*-Pinocarvil acetate	0.10±0.01	1295	1300	1661	1661	1,2	Monoterpenoid ester
40	*cis*-Pinocarvil acetate	0.25±0.04	1304	1308	-	-	1,2	Sesquiterpenoid alcohol
41	δ-Elemene	0.35±0.03	1320	1324	1469	-	1,2	Monoterpenoid ester
42	(Z)-Patchenol	0.63±0.11	1340	1345	-	-	1,2	Sesquiterpenoid alcohol
43	β-Cubebene	0.26±0.03	1351	1349	1542	1457	1,2	Sesquiterpene hydrocarbon
44	(E)-Patchenol	2.71±0.21	1354	1356	-	-	1,2	Sesquiterpene hydrocarbon
45	*cis*-Carvyl acetate	0.73±0.03	1365	1362	-	1782	1,2	Sesquiterpene hydrocarbon
46	α-Copaene	0.19±0.01	1376	1375	1491	1500	1,2	Monoterpenoid ketone
47	Daucene	0.58±0.07	1380	1378	1495	-	1,2	Sesquiterpene hydrocarbon
48	β-Longipinene	0.11±0.04	1408	1405	-	1482	1,2	Sesquiterpene hydrocarbon
49	β-Cedrene	0.12±0.02	1423	1424	-	1620	1,2	Sesquiterpene hydrocarbon
50	Aromadendrene	0.12±0.03	1439	1438	1620	1640	1,2	Sesquiterpene hydrocarbon
51	*allo*-Aromadendrene	0.22±0.02	1458	1457	1660	1637	1,2	Sesquiterpene ester
52	Germacrene D	0.40±0.05	1480	1476	1708	1708	1,2	Sesquiterpene hydrocarbon
53	Chrysantenyl 2-methylbutanoate	0.35±0.04	1488	1489	-	-	1,2	Monoterpenoid ester
54	Bornyl 2-methylbutanoate	0.08±0.01	1489	1488	1241	-	1,2	Monoterpenoid ester
55	δ-Amorphene	0.19±0.02	1508	1511	-	1772	1,2	Sesquiterpenoid alcohol
56	Palustrol	0.27±0.03	1546	1550	1930	1931	1,2	
57	Spathulenol	1.25±0.17	1572	1572	2127	2224	1,2	Sesquiterpenoid alcohol
58	Caryophyllene oxide	0.74±0.11	1573	1578	1986	1989	1,2	
59	Viridiflorol	0.78±0.14	1586	1588	2090	2100	1,2	Sesquiterpenoid alcohol
60	Junenol	0.06±0.01	1629	1627	-	-	1,2	Sesquiterpenoid alcohol
61	γ-Eudesmol	0.32±0.01	1630	1629	2176	-	1,2	Sesquiterpenoid alcohol
62	Caryophylla-4(12),8(13)-dien-5α-ol	0.12±0.02	1637	1639	2324	2324	1,2	Sesquiterpenoid alcohol
63	Isospathulenol	0.16±0.02	1640	1644	2228	2224	1,2	Sesquiterpenoid alcohol
64	τ-Cadinol	0.23±0.02	1645	1649	2151	2166	1,2	Sesquiterpenoid alcohol

Table 2 ***cont***.

No.	Compound	%	Ki[a]	Ki[a1]	Ki[b]	Ki[b1]	Identification[c]	Chemical class
65	α-Eudesmol	0.34±0.04	1654	1652	2250	2250	1,2	Sesquiterpenoid alcohol
66	α-Cadinol	0.58±0.04	1656	1655	2224	2221	1,2	Sesquiterpenoid alcohol
67	(1R,7S)-Germacra-4(15),5,10(14)-trien-1β-ol	0.16±0.02	1684	1686	-	-	1,2	Sesquiterpenoid alcohol
Total		95.49±0.24						
Monoterpene hydrocarbons		9.66±0.85						
Oxygenated monoterpenes		79.27±1.23						
Sesquiterpene hydrocarbons		2.14±0.13						
Oxygenated sesquiterpenoids		8.70±0.46						

[a,b]Kovats retention indices determined relative to a series of *n*-alkanes (C10–C35) on the apolar HP-5 MS (Ki[a]) and the polar HP Innowax capillary columns(Ki[b]), respectively; [a1,b1]Kovats indices from literature; [c]Identification method: 1 = comparison of the Kovats retention indices with published data, 2 =comparison of mass spectra with those listed in the NIST 02 and Wiley 275 libraries and with published data; 3 = co-injection with authentic compounds; The results are the mean of three experiments ± SD

index: -0.91±0.05; $\chi^2_{(1)}$= 39.3, $p < 0.001$). Subsequently, the behavioural response of *D. suzukii* to the experimental trials was evaluated (Fig. 1).

A strong repellent activity against *D. suzukii* was observed for the carvacrol ($\chi^2_{(1)}$ = 25.3, $p < 0.001$) and at a lower level for the thujone ($\chi^2_{(1)}$ = 5.54, $p < 0.05$) and camphor ($\chi^2_{(1)}$ = 5.83, $p < 0.05$), whereas the EOs had no significant effects ($\chi^2_{(1)}$ = 1.67 and $\chi^2_{(1)}$ = 2.7, respectively). For each compound, the differences among replicates were never found to be significant.

For the two-choice trap assay, the method was first validated by placing only apple cider vinegar in both traps (positive control), confirming that in the absence of differential attractants, adult *D. suzukii* showed no preference between traps (RI= -0.04±0.07; $\chi^2_{(1)}$ = 0.074, p = 0.79). The results of the two-choice trap assays performed to test whether the compounds reduce *D. suzukii* attraction to the apple cider vinegar are shown in Fig. 2.

When combined with the attractant, we found a strong repellent activity against *D. suzukii* for the *A. herba-alba* EO, thujone, and carvacrol ($\chi^2_{(1)}$ = 22.9, $\chi^2_{(1)}$ = 17.3, and $\chi^2_{(1)}$ = 46.2 respectively; $p < 0.001$ in all cases), a moderate repellent activity for the camphor ($\chi^2_{(1)}$ = 8.53, $p < 0.01$), whereas *O. vulgare* EO showed a strong attractive activity ($\chi^2_{(1)}$ = 40.7; $p < 0.001$). For each compound, the differences among replicates were never found to be significant.

Oviposition bioassays

In the oviposition choice bioassays, *D. suzukii* laid more eggs in the *O. vulgare* EO treated diet compared with the control diet ($t_{(18)}$ = 2.19, $p < 0.05$). The differences in the number of eggs laid in the remaining treated medium compared with the control diet were all not significant ($p > 0.1$ in all cases, Fig. 3).

The results of the oviposition no-choice assay showed a significant effect of the treatment on the mean number of eggs laid by *D. suzukii* ($F_{5,36}$ = 3.95, $p < 0.05$), while no significant differences were found between replicates.

In this assay, the number of eggs laid by a single female was reduced only in the carvacrol treated medium compared with the control (Tukey HSD test), while the mean number of eggs laid in the other groups was not statistically different from each other (Fig. 4).

Discussion

This study provides evidence of the behavioural effects of plant-derived EOs and their main constituents on *D. suzukii*, an insect pest that causes significant economic damage to soft fruits[7]. The use of EOs from *O. vulgare* and *A. herba-alba*, along with their main components, was evaluated through a series of bioassays investigating repellency and oviposition deterrence. Our findings highlight the importance of testing both whole EOs and individual constituents, as interactions within complex mixtures can alter their biological activity.

The chemical composition of *O. vulgare* EO has been studied, revealing significant variability due to the high intraspecific diversity of this plant[31]. Several chemotypes have been reported, the most common of which are carvacrol (to which the EO of this study belongs), thymol, linalool, α-terpinene, and *p*-cymene[31]. These findings demonstrate that monoterpenes are the predominant class of compounds[32]. The main components reported are carvacrol, thymol, linalool, γ-terpinene, *p*-cymene, β-caryophyllene, and germacrene[11,32]. Many of these compounds were present in the EO used in this study, with carvacrol being the main component (67.11±0.76%), followed by *p*-cymene (18.03±0.41%). α-Terpinene,

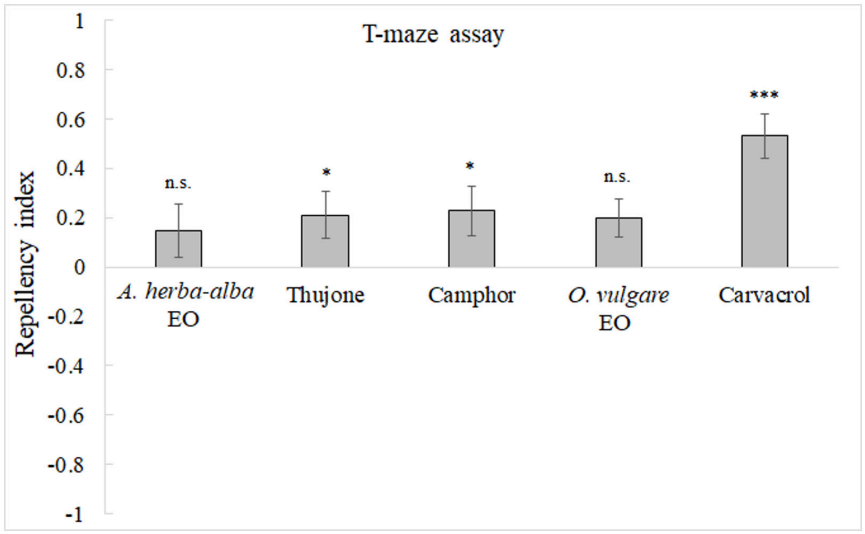

Figure 1. Avoidance behaviour assessed using the T-maze assay, where insects choose between two odor cues to evaluate attraction or repellency. Mean repellency index values (± standard errors) of *Drosophila suzukii* females in response to the tested compounds were calculated as (C − O) / (C + O), where C is the number of flies in the control trap and O is the number of flies in the test odorant compound trap. Statistically significant *P*-values, determined using Chi-square tests, are marked with asterisks (n.s.: not significant; *: $p < 0.05$; ***: $p < 0.001$)

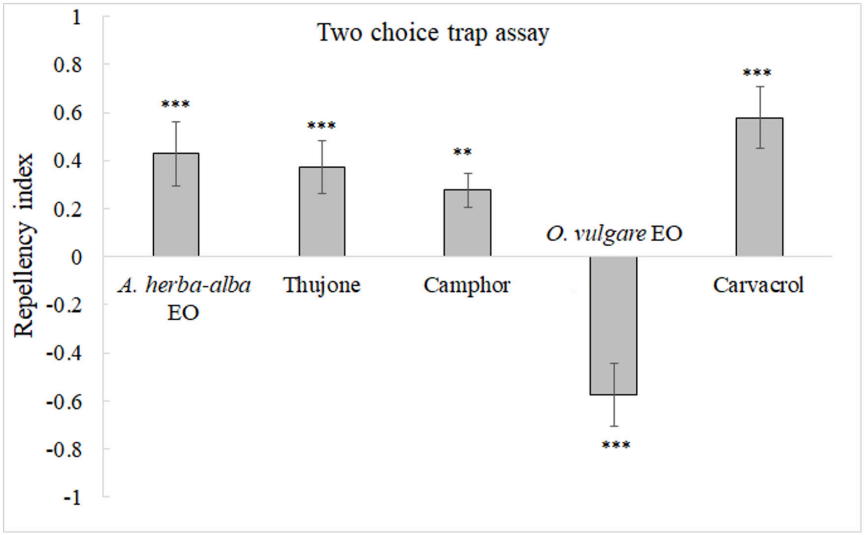

Figure 2. Avoidance behaviour in the two-choice trap assay. Mean repellency index values (± standard errors) of *Drosophila suzukii* females in response to the tested compounds were calculated as (C − O) / (C + O), where C is the number of flies in the control trap, and O is the number of flies in the test odorant compound trap. Statistically significant *P*-values, determined using Chi-square tests, are marked with asterisks (**: $p < 0.01$; ***: $p < 0.001$)

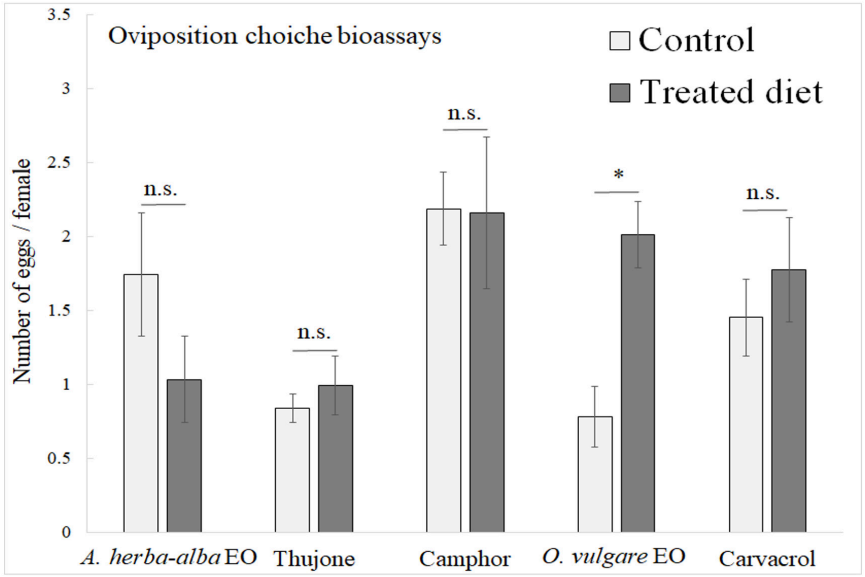

Figure 3. Oviposition choice assay showing the mean number of eggs laid (± standard errors) by a single *Drosophila suzukii* female when given a choice between control and treated diet. Statistically significant *P*-values, determined using Chi-square tests, are indicated by asterisks (n.s.: not significant; *: $p < 0.05$)

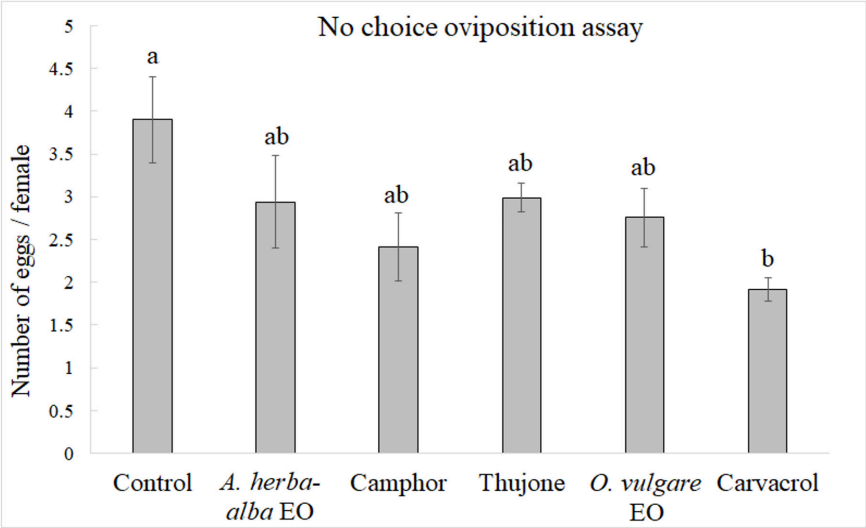

Figure 4. Results from the oviposition no-choice assay. Mean number of eggs laid (± standard errors) by a single *Drosophila suzukii* female confined to a diet treated with a single compound. Means with different superscript letters indicate significant differences (Tukey test, α = 0.05)

γ-terpinene, thymol, and β-caryophyllene have also been detected but all in very low amounts (0.55-1.21%). EOs of *O. vulgare* from Northern Italy are typically characterized by the presence of γ-terpinene (1.82-18.20%), linalool (0.58-28.18%), thymol (0.72-21.65%), carvacrol (0.14-18.47%), γ-muurolene (3.99-10.48%), germacrene-D-4-ol (0.33-10.69%), and caryophyllene oxide (0.60-15.82%)[33]. In contrast, EOs from southern Italy are generally rich in γ-terpinene (0.30-31.30%), thymol (0.20-61.50%), and carvacrol (0.20-70.80%)[34]. The composition of the EO analyzed in this study is consistent with the profiles reported for Italian *O. vulgare*, particularly those originating from southern regions, where carvacrol is commonly identified as the predominant component.

The EO of *A. herba-alba* is characterized by a great diversity in composition, depending on the country in which it was found and sometimes even in different locations in the same country[27,29]. However, all the works agree in reporting monoterpenoids as the main components, especially the monooxygenated ones such as 1,8-cineole, chrysanthenon, chrysanthenol, camphor, and α- and β-thujones[35].

The EO of this work is also in agreement with this, having a predominance of oxygenated monoterpenes (79.27±1.23%) of which camphor (26.22±0.67%), α- and β-thujone (9.85±0.63 and 9.17±0.71%, respectively) were the major components.

In Tunisia, four chemotypes are present (*trans*-sabinyl acetate, α-thujone/*trans*-sabinyl acetate, camphor, and α-thujone/camphor/β-thujone) and oxygenated monoterpenes were found to be the predominant class[36]. Among them, the main ones are 1,8-cineole, thujones, chrysanthenone, camphor, borneol, chrysanthenyl acetate, and sabinyl acetate[17,36]. The EO analyzed in this study is consistent with previous reports, corresponding to the camphor chemotype. The EO also presents most of the main compounds reported in literature (camphor, 1,8-cineole, α-thujone, β-thujone, chrysantenone, and borneol), albeit with quantitative variations. Finally, the EO of this work presented similarities, with quantitative variations, also with EOs found in other countries. Oxygenated monoterpenes and compounds such as camphor α-and β-thujone, 1,8-cineole, camphene, chrysanthenone, chrysanthenol, and davanone are characteristic of EOs from Algeria[37], Morocco[38], Spain[39], Egypt[40], and Middle East[41]. The contributions examined regarding the chemical composition also provide information on the EO yield (%) of the two plants, which allow a comparison with those obtained for the EOs of this work. As regards the EO of *O. vulgare*, the yield obtained in this work (1.8%) is perfectly in line with the yields reported for EOs obtained in other locations, which settle in a range between 0.54 and 5.10%[31-34]. However, the EO yield of *A. herba-alba* is much lower, which in this work is 0.1%, while at a global level higher yields have been recognised, ranging from 0.41 to 2.30%[17,35,37-39,41,43,44]. It is known that the chemical composition of an EO is subject to variability due to intrinsic factors (species, chemotype, age, and part of the plant) and extrinsic factors (geographical origin, environmental conditions, harvesting season, and extraction method)[42]. This variability inevitably influences the biological properties of EOs, including their repellency and insecticidal activity, as these effects are largely attributed to specific compounds (such as carvacrol, thujone, cymene, and camphor), whose relative abundance may vary according to the factors described above[43,44]. It is therefore essential to consider these variations and to chemically characterize each EO obtained and used in biological assays. This compositional variability emphasizes the importance of evaluating individual compounds, as key constituents are likely responsible for the observed behavioral effects. Our findings align with recent reports demonstrating the larvicidal activities of *Artemisia* spp. EOs against the mosquito *Culex pipiens* are

closely linked to their oxygenated monoterpene content, with camphor, thujones, and chrysanthenone emerging as key bioactive constituents[44]. This reinforces the importance of chemotypic variability in determining biological efficacy and supports the evaluation of individual compounds in addition to whole oils. While the whole EOs of *O. vulgare* and *A. herba-alba* did not alter fly distribution, analysis of their main constituents revealed specific repellent or attractive effects, highlighting the importance of testing individual compounds and supporting previous reports of intra-mixture antagonistic or synergistic interactions[13,15,26]. However, the main constituents of these EOs, carvacrol, α-thujone, and camphor, produced strong repellent responses. In the two-choice trap assay, *A. herba-alba* EO and its main constituents (α-thujone and camphor) were found to significantly reduce the attraction of *D. suzukii* to apple cider vinegar, thus confirming their repellent efficacy. Conversely, *O. vulgare* EO exhibited a significant attractant effect. Carvacrol, which acts as a repellent when tested as a pure compound, may have reduced or masked effect when present within the complex mixture of *O. vulgare* EO, as a result from interactions with other constituents in the EO. This differential activity suggests that isolated compounds may produce more consistent behavioural effects than whole EO, which may contain components with opposing activities due to their complex chemical matrices. These results are consistent with previous studies indicating that the biological efficacy of EOs may be altered by intra-mixture synergistic or antagonistic effects among components[13,15,26,45].

The mechanisms underlying the repellent and insecticidal activity of the main compounds considered have been addressed in various studies. The repellency exerted by camphor appears to be linked both to its ability to cause sensory effects (olfactory sensory alterations) and to irritant and deterrent contact actions[46]. α- and β-Thujone, on the other hand, have a modulatory action on GABA receptors and chloride channels, causing the onset of neurotoxic effects[47]. Carvacrol also targets the nervous system, acting on chloride channels and inhibiting acetylcholinesterase activity[48]. Finally, *p*-cymene seems to act as a synergist, promoting penetration and positively influencing the bioavailability of other compounds[49].

O. vulgare EO stimulated oviposition, consistent with prior findings on the use of attractant plant extracts to manipulate *D. suzukii* behavior[50]. Together with the attractive activity observed in the two-choice trap assay, this result suggests the potential application of *O. vulgare* EO within an IPM framework, as part of an "attract-and-kill" or "lure-and-contaminate" strategy[50]. The EO could enhance the attractiveness of artificial oviposition sites (e.g., baited traps), thereby diverting females away from host crops. Incorporating insecticidal agents into these traps would allow targeted pest suppression, reducing reliance on broad-spectrum insecticides and minimizing impacts on non-target organisms. This strategy aligns with the principles of sustainable and environmentally friendly pest management, supporting the development of EO-based repellents and attractants for IPM, organic farming, and potential commercial products, highlighting the importance of identifying specific active constituents.

In contrast, both *A. herba-alba* EO and, more notably, its primary constituent α-thujone, significantly inhibited oviposition. These observations suggest that α-thujone may interact with the chemosensory mechanisms that underlie host substrate recognition and oviposition site selection. This result is consistent with previous reports describing similar effects for thujone-rich plant extracts[13]. The efficacy of carvacrol, α-thujone, and camphor in repellency and oviposition deterrence tests suggests that selectively modifying the behaviour of *D. suzukii* by applying these components is possible. These results are consistent with previous studies that have demonstrated the insecticidal, repellent, and developmental inhibitory activities of α-thujone and carvacrol against various dipteran pests[12,14,18,51]. Taken together, these findings suggest that *A. herba-alba* EO, camphor, α-thujone, and carvacrol may reduce the attraction of *D. suzukii* to ripened fruit. Testing individual EO components allows for the identification of consistent behavioral effects, highlights promising candidates for sustainable pest management, and informs the development of eco-friendly botanical products compatible with IPM and organic farming.

Conclusions

With increasing restrictions on synthetic insecticides and growing consumer demand for environmentally friendly pest control methods, products based on the EOs of *A. herba-alba* and *O. vulgare* represent a promising solution for sustainable integrated pest management of *D. suzukii*. Our results showed that there is divergence in responses to EOs and single compounds, which reinforces the necessity of characterising the chemical profiles of EOs and contextualising their application in order to accurately predict efficacy. Specifically, α-thujone and camphor from *A. herba-alba* exhibited strong repellent and oviposition-deterring effects, while carvacrol showed consistent repellency as a pure compound, and *O. vulgare* EO demonstrated attractant activity. Future research should expand upon these preliminary results by conducting field trials to confirm the efficacy of EO-based biopesticides under real-world conditions and optimizing their formulations for practical use. Additionally, studies should assess the potential effects on non-target organisms and evaluate possible synergistic or antagonistic interactions among EO components. This will ensure the safe and effective deployment of EO-based biopesticides. Taken together with recent studies on *Artemisia* EOs, our results further highlight that differences in EO yield, and composition can

translate into distinct biological properties, underlining the need for chemotype-specific evaluations and formulation strategies to ensure consistent efficacy in pest management applications.

Statement of conflicting interests

The authors state that there is no conflict of interest.

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