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ORIGINAL PAPER

Essential oils from two aromatic plants repel the tobacco whitefly *Bemisia tabaci*

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Abstract

Characterizing the olfactory responses of insect pests is critical for developing biological control options and pest management strategies in the field. Such responses form the basis for evaluating interactions between plants and insects, as well as providing evidence to support the use of non-crop plant types in pest suppression tactics. To evaluate the potential aversion or attraction of *Bemisia tabaci* MED/Q to volatiles from various plants, behavioral responses of adult whitefly were observed in Y-type olfactometer tests ($n = 30$ individuals per trial, with 3 replicate trials per treatment combination; $n = 4230$ individuals in total). We quantified the potential repellent effects of the essential oils of *Thymus pulegioides* ('thyme') and *Artemisia absinthium* ('wormwood') on *B. tabaci* MED/Q. The essential oil of *T. pulegioides* as well as three of the four major subcomponents tested (thymol 36.18%, *p*-Cymene 10.85% and thymol methyl ether 7.45%, but not Carvacrol 13.43%) had significant repellent effects on *B. tabaci* MED/Q. Similarly, the essential oil of *A. absinthium*, as well as the two major subcomponents tested (Linalool 23.41% and (−)-β-Pinene 27.88%), had marginally significant repellent effects on *B. tabaci* MED/Q. In tests across increasing concentrations of these volatile compounds, repellent effects were typically only significant at the two highest concentrations tested. Overall, these results demonstrate that major constituents of certain aromatic plant oils have a strong repellent effect and contact toxicity on *B. tabaci* MED/Q. These findings have important implications for more environmentally friendly biological control options using aromatic plants to repel target pests in production crops.

Keywords *Thymus pulegioides* · *Artemisia absinthium* · Behavioral response · Olfaction · Volatile · *Bemisia tabaci* MED/Q

Key message

- Aromatic plants thyme and wormwood have potential to be effective repellents for whiteflies.
- The constituents of essential oils had repellent activity and contact toxicity on whiteflies.
- The results provide new aromatic plant products for environmentally friendly pest control.

Introduction

Plant volatiles provide an important basis for host selection by herbivorous insects, and during the course of evolution, plants have produced repellent volatile substances to deter feeding damage (De Moraes et al. 2001; Paré and Tumlinson 1999). With recent advances in phytochemical separation and purification technology (Altemimi et al. 2017), the potential use of plant essential oils for pest management has

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attracted increasing attention (Chaieb et al. 2018; Benelli et al. 2019a; Benelli et al. 2019c; Ikbal and Pavela 2019; Soares et al. 2019; Peres et al. 2020; Shah et al. 2020), particularly in the context of feeding-deterrent mechanisms for serious agricultural pests (Isman 2020). Insect olfactory responses to plant volatiles will be helpful in testing the viability of using non-crop plant types in pest suppression tactics.

The silverleaf whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae), is one of the most devastating agricultural pests worldwide, primarily in tropical and subtropical habitats. It not only causes direct feeding damage from sap sucking, but also causes indirect damage as an effective vector of up to 100 plant pathogenic viruses (Taggar and Gill 2016). The highly invasive *B. tabaci* Mediterranean (MED) form, also known as biotype Q or MED/Q, predominates in many regions of the world and causes severe crop damage and economic losses in countries such as China and India (Kumar et al. 2019; Rao et al. 2011). *Bemisia tabaci* feeds mainly on the undersides of crop leaves, where it is not readily affected by conventional spraying of insecticides. Consequently, farmers must use excessive applications of chemical insecticides in order to control populations. As a result, there have been widespread reports of insecticide resistance in *B. tabaci* MED/Q in recent years that have weakened the prospects for effective management using conventional practices (Yao et al. 2017; Zheng et al. 2017). There is currently a urgent need to identify effective natural alternative products and environmentally friendly pest control methods, with a lower risk to the environment and non-target species than the chemical pesticides widely used worldwide (Desneux et al. 2007; Khederi et al. 2019). The application of aromatic plant oils has been studied extensively for use in pest control, including their application against *B. tabaci* MED/Q, as potential alternatives to synthetic pesticides. Using these aromatic plants as a component of habitat manipulation strategies within agroecosystems has been identified as one of the most effective solutions in conservation biological control (CBC) (Carvalho et al. 2017; Gurr et al. 2017; Hatt et al. 2019; Khan et al. 2008).

Thymus pulegioides L. (Lamiaceae) is widely distributed in Mediterranean regions of Europe and typically occurs in meadows and on undisturbed soils. This species is less commercially important than other thyme species, such as *T. vulgaris* and *T. zygis* (Lawrence 1992). However, it has known insect repellent properties, with one constituent thymol being an active ingredient in pesticide products registered for use as insecticides, animal repellents and fungicides (Ansari et al. 2010; Bekircan et al. 2014; Park et al. 2017). Moreover, *T. pulegioides* has been used for its antiseptic and anti-inflammatory properties and has been reported to have antioxidant action, lipid peroxidation inhibition and free radical scavenging activity (Fernandes et al. 2010). Similarly, *Artemisia* species (Asteraceae) are known for their pharmacological, repellent, antifeedant and insecticidal properties (Bachrouch et al. 2015; Negahban et al. 2006, 2007; Schmitz 1999). *Artemisia absinthium* L., is a small perennial shrub that has been used as an herbal medicine throughout Europe, North America, Asia and South Africa (He et al. 2009). *A. absinthium* oil exhibited strong fumigant toxicity against *Rhyzopertha dominica* (Coleoptera: Bostrichidae) adults, a stored product pest and high fumigant activity against *Spodoptera littoralis* (Lepidoptera: Noctuidae), a greenhouse pest (Dhen et al. 2014). Previous studies have shown the repellent and anti-ovipositional effects of the essential oils of *T. vulgaris* and *A. camphorata* on several species of whitefly including *Trialenrodes vaporariorum* (Westwood), *B. tabaci* (MEAM1, also called biotype B) and *Aleuroclava jasmini* (Takahashi) (Aroiee et al. 2005; Baldin et al. 2013; Khederi et al. 2019; Yang et al. 2010). *A. absinthium* oil possesses antitrypanosomal, antiplasmodial, analgesic and antidepressant and antioxidant (Tariku et al. 2011).

Given the importance and widespread availability of these two plant species, and their potential use in conservation biological control, the primary objective of our research was to evaluate the repellent effectiveness of plant volatile extracts of *T. pulegioides and A. absinthium*, as well as their main constituent chemical components, and contact toxicity against *B. tabaci* MED/Q. We then tested the sensitivity of *B. tabaci* responses to varying concentrations of the major volatile components. Such information will be important in the context of developing more environmentally friendly pest management strategies for *B. tabaci* MED/Q.

Materials and methods

Plant material

The *T. pulegioides* plants used for the extraction of essential oils were collected from a commercial grower at Hulun Buir State Farm (49º12′ N, 119º73′ E, Hulun Buir, China). The *A. absinthium* plants were collected from the aromatic plant garden at the Institute of Botany, Chinese Academy of Sciences (Beijing, China).

Insect collection and rearing

A population of *B. tabaci* was supplied by the Institute of Plant & Environment Protection, Beijing Academy of Agriculture and Forestry Science. They were reared on cotton (*Gossypium hirsutum* L. var. 'Shiyuan 321') seedlings in ventilated mesh cages in a greenhouse compartment (26 ± 2 ℃, RH 60 – 70%, L:D = 14:10 photoperiod). The biotype of the *B. tabaci* population was confirmed using a cleaved-amplified polymorphic sequence marker for cytochrome oxidase I (mtCOI), as described by Khasdan et al. (Khasdan et al. 2005). PCR amplification was performed using primers C1-J-2159 and L2-N-3014 (Frohlich et al. 1999), which was followed by digestion with VspI, generating a clear polymorphism biotype MED/Q.

Extraction of essential oils, gas chromatography and mass spectrometry

When flowering (mid-July 2016 for *T. pulegioides* and late-August 2016 for *A. absinthium*) the aboveground parts of plants (including flowers, stems and leaves) were collected, air-dried and finely ground and subjected to 3 h steam distillation using a Clevenger-type apparatus (British type), according to the procedure described in the European Pharmacopoeia (Anonymous 1996) to produce the essential oils in a yield of 0.27% and 0.67% (v/w) based on the dry weight of the samples from *T. pulegioides* and *A. absinthium*, respectively. The distilled essential oils were dried over anhydrous sodium sulphate, and after filtration, stored in sealed vials at 4℃ until further analysis.

The essential oils were analyzed by gas chromatography–mass spectrometry (GC–MS) techniques. GC–MS analysis was carried out using a gas chromatograph Agilent-Technologies 7890 N (Agilent-Technologies, Palo Alto, CA, the USA) equipped with a split-splitless injector, Agilent-Technologies 7683 autosampler and an Agilent HP5-MS fused silica column (5% phenyl-methylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 µm). Injector temperature was 250℃, and the heating program was as follows: 40 °C for 2 min, linear ramp at a rate of 4 ℃ min^{-1} to 260 ℃, and then ramp to 310 ℃ at 60 ℃ min^{-1}, before being held at 310℃ for 15 min. The transfer line temperature was 280 ℃. Helium was used as carrier gas at a flow rate of 1.0 mL min^{-1} through the column. Samples were diluted in hexane with a ratio of 1:100, and 1 µL was injected in the split mode (1:20). The GC was fitted with a quadrupole mass spectrometer with Agilent-Technologies 5973 detector. The MS conditions were as follows: ionization energy, 70 eV; electronic impact ion source temperature, 200 °C; quadrupole temperature, 150 °C; mass range, 40–600 u. Agilent masshunter 5.0 was used to analyze the mass spectra and chromatograms.

Identification of components

Most constituents of the essential oils were identified by comparison of their linear retention indices (RIs) with values in the literature. The linear retention indices were determined by injection of a hexane solution containing the C7–C40 series of *n*-alkanes under the same operating conditions. Further identification was made by comparison of their mass spectra with either those stored in the mass spectra databases (NIST, ver. 8.0 and Wiley 275) or with mass spectra from a home-made library. The relative concentrations of components were obtained by peak area normalization. No response factors were calculated.

Y-tube olfactometer behavioral experiments

The experiments were conducted in a dark laboratory at 26 ± 2 ℃, RH 70 ± 5%. In all experiments, the responses of the whiteflies to volatiles were tested in a closed system Y-tube olfactometer (internal diameter, 2.5 cm; stem length, 15 cm; arm lengths, 10 cm at 75° angle) (Takabayashi and Dicke 1992). A 20 W warm yellow LED located above the device provided uniform lighting. Two streams of purified air filtered through activated charcoal were each blown through a 100 mL glass container and into the arms of the olfactometer at 100 mL min^{-1}. The base of the olfactometer was connected to a house vacuum at 200 mL min^{-1}. Prior to the introduction of each whitefly, a 1 × 2 cm rectangular filter paper impregnated with 1 µL of either the treatment chemical or a solvent control solution (paraffin) was inserted into each Y-tube arm odor source container. Whiteflies were starved for 4 h in advance of the olfactometer test, and one adult was individually introduced at the basal end of the Y-tube. Each whitefly adult was used only once in a single test. Whitefly behavior was observed, and the odor choice was recorded when it passed a marker point half way up either one of the arms of the Y-tube. A 'no choice' was recorded when the whitefly did not reach the marker point within 5 min, and these data were not used in analyses. Whiteflies were tested until 30 successful choice tests had been completed for each treatment comparison. Odor sources were interchanged between the arms of the Y-tube after every five whiteflies testing, in order to account for the influence of unforeseen asymmetric aspects of the Y-tube setup. The olfactometer and glass containers were cleaned and rinsed with EtOH and deionized H_2O after each set of 30 successful choice tests had been completed.

Odor sources

New filter papers with odor sources or paraffin were used for each individual whitefly. Odor sources in the experiments included the extracted essential oils of *T. pulegioides* and *A. absinthium*, as well as identified subcomponents of each essential oil: thymol 36.18%, carvacrol 13.43%, *p*-Cymene 10.85% and thymol methyl ether 7.45% were the main components of the essential oil of *T. pulegioides*. The main components of essential oil *A. absinthium* include (−)-β-Pinene 27.88% and Linalool 23.41%. Five concentrations (0.01, 0.1, 1, 10 and 100 µg) of thymol, carvacrol, *p*-Cymene, or linalool dissolved in 0.1 mL paraffin were applied in behavioral experiments. Similarly, 0.01, 0.1, 1, 10 and 100µL of thymol methyl ether, or (−)-β-Pinene diluted in 0.1 mL paraffin were used in the behavioral experiments. All compounds were commercially available from Sigma-Aldrich. Each experiment (odor source vs control) was repeated three times (i.e., 3 × 30 starved whiteflies) for a particular concentration of odor source on three different experimental days, with a new whitefly tested on each occasion (4230 successful whitefly choice tests in total).

Contact toxicity assays

The bioefficacy of essential oil components against adults of *B. tabaci* was evaluated on following the methods of Saad et al. 2017. The essential oils were diluted in paraffin, to achieve a concentration of 0.01, 0.1 and 0.5 (ml/L total volume). Fresh leaves from tomato plant were secured with cotton swab immersed in 10% sucrose solution and agar and put in the transparent 300-mL plastic cup. Each solution was applied to filter paper (7-cm diam) by using a micropipette, with 5 µl of essential oil, or with paraffin (control 1) or with distilled water (control 2).The filter paper was dried under a ventilated hood before attaching to the inside of a 7-cm culture plate. Nearly 20 adult whiteflies were introduced into cup, and the culture plate with the treated filter paper was covered. *B. tabaci* adult mortality was recorded after 1 h, 3 h, 5 h and 24 h later. (When no leg or antennal movements were observed, the insect was considered dead.) Treatment concentrations were replicated 12 times.

Statistical analysis

We tested *B. tabaci* MED/Q responses to the essential oil of *T. pulegioides* (and its four major subcomponents: thymol 36.18%, *p*-Cymene 10.85%, carvacrol 13.43% and thymol methyl ether 7.45%) as well as the essential oil of *A. absinthium* (and its two major subcomponents of (−)-β-Pinene 27.88% and linalool 23.41%) and compared these to the control response for paraffin alone, using a generalized linear mixed effects model (GLMM) in the 'glmmTMB' package (Brooks et al. 2017) in R 3.6.2 (R Core Team, 2019). We specified a binomial error structure (with logit link function) and specified random effects in the model for 'Trial' (to account for potential non-independence of replicate individuals tested in the same batch of 30 test runs), 'Date' (to account for potential non-independence of multiple cohorts of individuals tested on the same day), and 'Side' (to account for idiosyncratic differences in lighting or other conditions on different sides of the Y-tube arms).

The same model specification was used for testing *B. tabaci* MED/Q responses to increasing concentrations of volatile compounds (0, 0.01, 0.1, 1, 10 and 100 µg or µL) for thymol, *p*-Cymene, carvacrol, thymol methyl ether, (−)-β-Pinene and linalool, except in this case fixed effects were specified in the model for the interaction between odor treatment and concentration.

The contact toxicity of essential oils on mortality of *B. tabaci* adult was analyzed by two-way repeated measures on general linear model. Means were separated when F text was significant on the basis of Tukey's test.

Results

B. tabaci responds to the essential oils of *T. pulegioides* and *A. absinthium*

When whiteflies were given the choice between a no odor control versus the odor of either one of the two essential oils, they significantly preferred the no odor control (Fig. 1, binomial GLMM, *T. pulegioides* $z = -4.621$, $P < 0.001$; *A. absinthium* $z = -5.231$, $P < 0.001$). In both cases, ca 80% of whiteflies tested were repelled by the volatile oils of *T. pulegioides* and *A. absinthium* (Fig. 1).

Chemical composition of the essential oils

A total of 44 different volatile compounds were detected in the essential oil of *T. pulegioides* (Table 1) and 33 volatile compounds in the essential oil of *A. absinthium* (Table 2). Of these, thymol (36.18%) was the main constituent of *T. pulegioides* essential oil, followed by carvacrol (13.43%), *p*-Cymene (10.85%) and thymol methyl ether (7.45%). For *A. absinthium*, the major constituents were (−)-β-Pinene (27.88%) and linalool (23.41%).

B. tabaci responds to the major volatile components within the essential oils of *T. pulegioides* and *A. absinthium*

Of the four major constituents of *T. pulegioides* oil, at their naturally occurring concentrations, only thymol and thymol methyl ether showed highly significant repellent action (> 70% of white flies repelled; $z = -3.783$ $P < 0.001$ and −3.259, $P < 0.01$), whereas *p*-Cymene had weaker, but significant, repellent effects (ca 65% of whiteflies repelled, $z = -2.546$ $P < 0.05$), while carvacrol had no significant effect relative to the solvent control (Fig. 1).

For the two major constituents of *A. absinthium* oil, at their naturally occurring concentrations, both (−)-β-Pinene and linalool had marginally significant repellent effects (ca 60% of whiteflies repelled, $z = -1.823$ and $z = -2.004$, $P < 0.05$), but at a much lower level of repellent action than the essential oil of *A. absinthium* (Fig. 1).

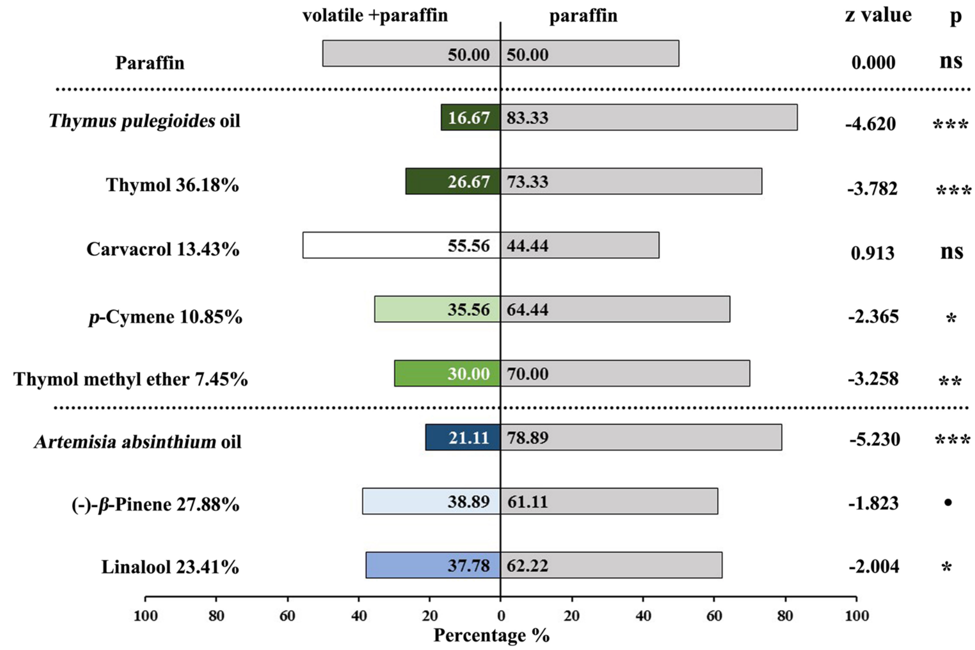

Fig. 1 *Bemisia tabaci* responses in Y-tube olfactometer tests to the odors of essential oils of *T. pulegioides* (including its four main constituents: thymol, carvacrol, *p*-Cymene and thymol methyl ether) and *A. absinthium* (including its two main constituents: (−)-β-Pinene and linalool) compared with control (paraffin) (ns, non-significant difference $P>0.1$; • $P<0.1$; *$P<0.05$; **$P<0.01$; ***$P<0.001$). The color's shades are for the different significant level. Green for *T. pulegioides* and blue for *A. absinthium*

Effects of varying concentrations of volatile components on B. tabaci MED/Q

The single-compound assays across an odor concentration gradient produced significant repellent effects on *B. tabaci* in all compounds except carvacrol (Fig. 2b). For components of *T. pulegioides* oil, responses were dose-dependent, and repellent action occurred at moderate to high concentrations of 1–100 µg for thymol, and at high concentrations of 10–100 µg for *p*-Cymene and thymol methyl ether (Fig. 2a, c, d). At the highest concentrations > 75–80% of whitefly were repelled, comparable to the essential oil effect for *T. pulegioides* (Fig. 1).

For components of *A. absinthium* oil, significant repellant effects were only seen at the highest concentration of 100 µg, for both (−)-β-Pinene and linalool (Fig. 2e, f). In particular, almost 75% of whiteflies were repelled at the highest concentration of (−)-β-Pinene, which is comparable to the essential oil effect for *A. absinthium* (Fig. 1). There was a weak, but non-significant, indication that whiteflies were attracted to the lowest odor dose of 0.01 µL linalool (62.22% of whiteflies attracted, $z=1.648$, $P<0.10$; Fig. 2f).

Contact toxicity against *B. tabaci* adults

Observed mortality 20 h after exposure to the *T. pulegioides* oils at concentrations of 0.01, 0.1 and 0.5 ml/L caused adult mortality of 53.39%, 69.18% and 84.02%, differed significantly with water and paraffin ($P<0.05$) (Table 3). The does 0.01, 0.1 and 0.5 ml/L of *A. absinthium* oil was statistically similar with *T. pulegioides* oil, killed 53.47%, 63.62% and 80.23% adults, respectively ($P<0.05$), when compared to the control (water and paraffin). As time goes on, the mortality of *B. tabaci* adults showed a significant increase when exposed to two essential oils at three concentrations ($F_{T.\ pulegioides}=282.63$, $F_{A.\ absinthium}=99.49$, all $df=4, 55$, $P<0.001$) (Table 4).

Discussion

Phytochemistry of aromatic essential oils

The essential oils of certain plant species can repel insect pests on crops, but their efficacy varies according to the phytochemical profile of the plant extract (Regnault-Roger et al. 2012). For example, different chemotypes of *T. vulgaris* have been shown to have different toxicities against the bruchid bean weevil *Acanthoscelides obtectus* (Regnault-Roger et al. 1993). Similarly, the curculionid maize weevil *Sitophilus zeamais* was found to be more sensitive to dominant compounds of farnesol (chemotype 1) or springene plus beta-farnesene (chemotype 2) from 'fish-poison bean' *Tephrosia vogelii* (Fabaceae), rather than to a mixture of the two chemotypes together (Kerebba et al. 2020). Therefore, the benefits of growing repellent plants or applying essential oil extracts can be highly variable in a crop production system depending on the chemotype used. In a management context, characterizing and isolating the dominant chemical constituents of highly repellent chemotypes could allow more efficient production and application of the active compound in pest control. While the repellent properties of essential oils are frequently attributed to the mixture ratio of constituents,

Table 1 Chemical constituents of the essential oil of *Thymus pulegioides*

Component	RI[lit]	RI[a]	Percentage of total (%)	Identification[b]
α-Thujene	925	919	0.12	1, 2
α-Pinene	938	924	0.14	1, 2
Camphene	953	939	0.18	1, 2
1-Octen-3-ol	980	973	0.53	1, 2
3-Octanone	992	980	0.11	1, 2
Myrcene	993	984	0.62	1, 2
α-Terpinene	1013	1009	0.18	1, 2
***p*-Cymene**	**1025**	**1017**	**10.64**	**1, 2**
Limonene	1030	1020	0.06	1, 2
1,8-Cineole	1034	1023	0.34	1, 2
γ-Terpinene	1057	1051	0.96	1, 2
trans-Sabinene hydrate	1093	1059	0.11	1, 2
Linalool	1097	1093	1.05	1, 2
Camphor	1145	1136	0.82	1, 2
endo-Borneol	1167	1157	1.06	1, 2
Terpinen-4-ol	1176	1169	0.66	1, 2
p-Cymen-8-ol	1185	1178	0.13	1, 2
α-Terpineol	1189	1183	0.17	1, 2
Thymol methyl ether	**1239**	**1228**	**7.36**	**1, 2, 3**
Carvacrol methyl ether	1245	1237	6.29	1, 2, 3
Thymol	**1293**	**1285**	**34.00**	**1, 2, 3**
Carvacrol	**1299**	**1294**	**11.74**	**1, 2, 3**
β-Bourbonene	1385	1377	0.19	1, 2
β-Caryophyllene	1415	1411	3.23	1, 2
allo-Aromadendrene	1463	1453	0.33	1, 2
γ-Muurolene	1478	1469	0.21	1, 2
Viridiflorene	1494	1487	0.20	1, 2
α-Muurolene	1503	1492	0.13	1, 2
β-Bisabolene	1510	1500	5.45	1, 2
γ-Cadinene	1515	1506	0.21	1, 2
δ-Cadinene	1526	1515	1.57	1, 2
trans-α-Bisabolene	1540	1535	1.27	1, 2
Caryophyllene oxide	1579	1575	0.62	1, 2
t-Cadinol	1640	1633	0.13	1, 2

RI indicates retention index. Bolded components indicate major constituents

[a]HP-5MS column

[b]1 = LRI linear retention index; 2 = MS identification based on comparison of mass spectra; 3 = co-injection/comparison with the LRI and mass spectra of authentic compounds

[lit] Literature (Adams 2007)

the likelihood in most cases is that the main components are probably responsible for the majority of the biological effects (Regnault-Roger et al. 2012). In this study, we report significant repellent action for the essential oil extracts of *T. pulegioides* in repellency class V and *A. absinthium* in repellency class IV (Jilani and Su 1983;

Table 2 Chemical constituents of the essential oil of *Artemisia absinthium.*

Component	RI[lit]	RI[a]	Percentage of total (%)	Identification[b]
α-Thujene	930	918	0.16	1, 2
α-Pinene	938	924	0.21	1, 2
Camphene	954	937	0.16	1, 2
Sabinene	977	965	15.16	1, 2
β-Pinene	**981**	**984**	**25.60**	**1, 2, 3**
α-Terpinene	1019	1009	0.41	1, 2
p-Cymene	1028	1017	0.34	1, 2
γ-Terpinene	1063	1051	1.02	1, 2
cis-Sabinene hydrate	1073	1059	0.17	1, 2
Linalool	**1100**	**1093**	**19.11**	**1, 2, 3**
trans-Sabinene hydrate	1110	1170	1.30	1, 2
Terpinen-4-ol	1186	1186	0.42	1, 2
α-Terpineol	1197	1194	0.31	1, 2
Verbenone	1205	1202	0.38	1, 2
Carvone	1222	1219	0.31	1, 2
1H-Indene, 1-ethylidene-octahydro-7a-methyl-, *cis*-	1239	1231	0.49	1, 2
Benzyanyl	1421	1379	0.17	1, 2
Caryophyllene	1434	1412	2.76	1, 2
β-Copaene	1442	1446	0.28	1, 2
Humulene	1455	1478	0.48	1,2
(+)-β-Selinene	1478	1502	1.46	1, 2
Germacrene D	1494	1506	3.73	1, 2
Dihydrochamazulene 1[c]	1518	1511	0.13	1, 2
Dihydrochamazulene 2[d]	1530	1569	1.35	1, 2
Elemol	1562	1576	0.61	1, 2
Spathulenol	1595	1595	0.63	1, 2
Caryophyllene oxide	1601	1609	1.83	1, 2
Dihydrochamazulene 3[e]	1634	1618	0.33	1, 2
β-Eudesmol	1649	1622	0.74	1, 2
α-Bisabolol	1670	1634	0.84	1, 2
γ-E-Atlantone	1706	1646	0.36	1, 2
Dihydrochamazulene 4[f]	1800	1992	0.40	1, 2
Chamazulene	1758	1720	1.33	1, 2
Gerany-*p*-cymene	1980	1999	2.57	1, 2

RI indicate retention index. Bolded components indicate major constituents

[a]HP-5MS column

[b]1 = LRI linear retention index; 2 = MS identification based on comparison of mass spectra; 3 = co-injection/comparison with the LRI and mass spectra of authentic compounds

[c]Isomer not determined

[d]Isomer not determined

[e]Isomer not determined

[lit]Literature (Adams 2007)

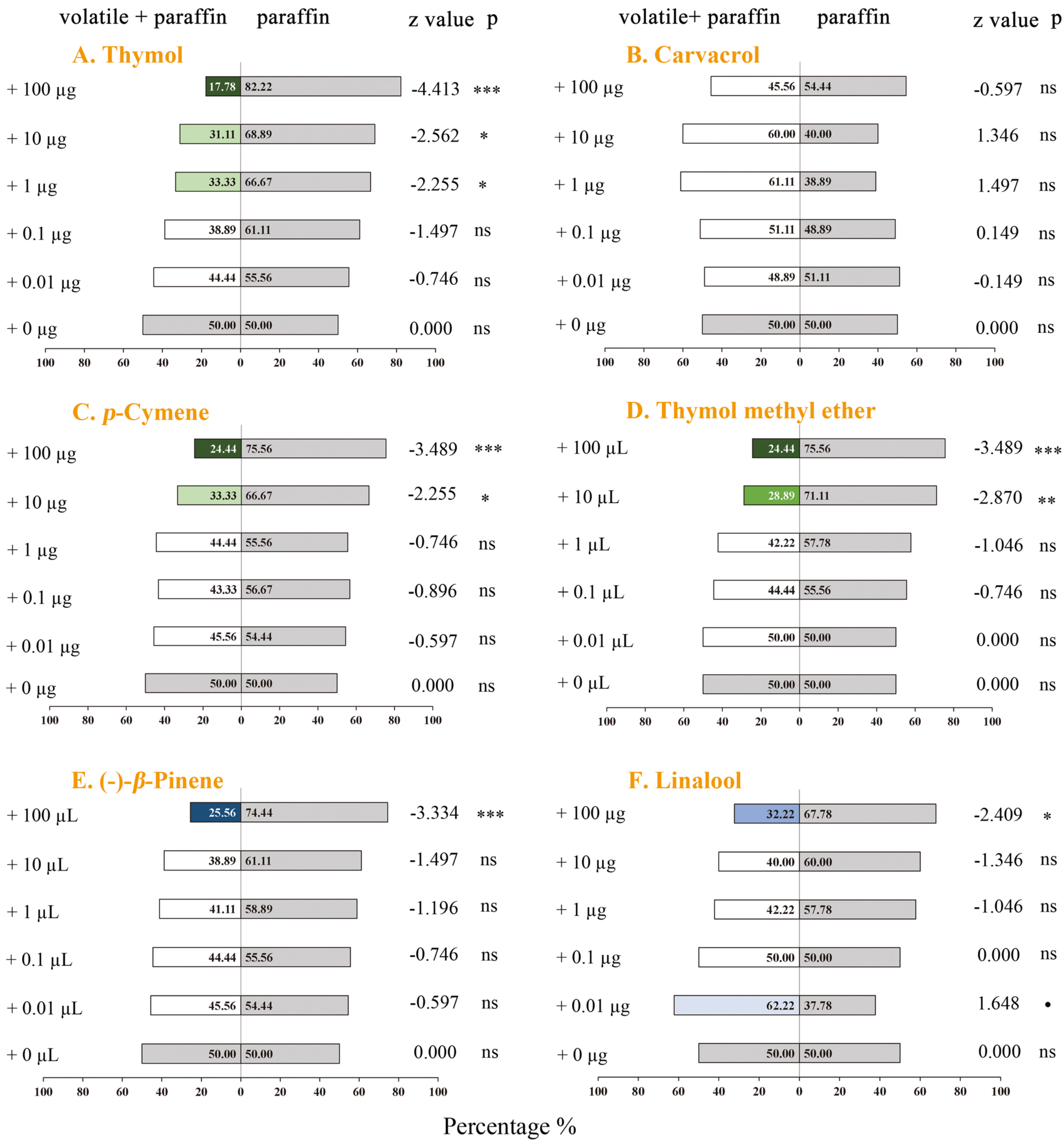

Fig. 2 *Bemisia tabaci* responses in Y-tube olfactometer tests to varying concentrations of odors of the major volatile components from *T. pulegioides* (**A**–**D**) and *A. absinthium* oil (**E**, **F**) compared with control (paraffin; indicated as 0 µg or µL concentration) (ns, non-significant difference $P > 0.1$ **F**; •$P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

Wagan et al. 2018). More importantly, we identify dominant constituents of the essential oils that have exceptionally promising repellent action at higher concentrations, including thymol, *p*-Cymene and thymol methyl ether (from *T. pulegioides* essential oil) and (−)-β-Pinene (from *A. absinthium* essential oil). We discuss the importance of these findings for more environmentally friendly conservation biological control measures in crop production systems.

Table 3 Percentage of mortalities (mean ± SE) of the adults of *Bemisia tabaci* exposed to *Thymus pulegoides* and *Artemisia absinthium* essential oils at different concentrations after different hours (replicates 12)

Concentration (mL/L)	Adult Mortality (mean ± SE) (%)*			
	1 h	3 h	5 h	20 h
Thymus pulegoides				
0.5	31.20% ± 1.34%^{a}	67.79% ± 2.37%^{a}	78.79% ± 1.55%^{a}	84.02% ± 1.19%^{a}
0.1	19.75% ± 0.99%^{b}	37.95% ± 1.87%^{b}	63.02% ± 1.98%^{b}	69.18% ± 2.01^{b}
0.01	16.88% ± 1.31%^{b}	27.45% ± 1.29%^{c}	38.29% ± 1.21%^{c}	53.39% ± 1.57%^{c}
Paraffin	7.21% ± 0.71%^{c}	8.46% ± 0.65%^{d}	12.35% ± 0.57%^{d}	12.76% ± 0.56%^{d}
Water control	2.80% ± 0.72%^{d}	5.71% ± 0.55%^{d}	6.50% ± 0.68%^{e}	8.16% ± 0.72^{d}
F	112.16	276.77	572.62	652.68
P value	< 0.001	< 0.001	< 0.001	< 0.001
Artemisia absinthium				
0.5	23.90% ± 1.24%^{a}	51.31% ± 1.09%^{a}	73.44% ± 2.27%^{a}	80.23% ± 2.23%^{a}
0.1	16.70% ± 1.52%^{b}	23.54% ± 3.05%^{c}	42.79% ± 3.69%^{b}	63.62% ± 3.06^{b}
0.01	16.40% ± 1.08%^{b}	33.22% ± 1.47%^{b}	46.25% ± 1.17^{b}	53.47% ± 1.42%^{c}
Paraffin	2.53% ± 0.76%^{c}	5.47% ± 1.14%^{d}	12.54% ± 1.47%^{c}	14.12% ± 1.27%^{d}
Water control	2.01% ± 0.72%^{c}	4.29% ± 0.89%^{d}	7.58% ± 0.97%^{c}	8.72% ± 1.17^{d}
F	75.95	132.3	157.56	253.82
P value	< 0.001	< 0.001	< 0.001	< 0.001

*Means followed by different letters (a/b/c) are significantly different treatments by the basis of Tukey's test. (p < 0.05)

Table 4 General linear model analysis of variance for effects of recorded time and treatments on the mortality of *Bemisia tabaci* exposed to *Thymus pulegoides* and *Artemisia absinthium* essential oils

	df	*Thymus pulegoides*		*Artemisia absinthium*	
		F	*P*	*F*	*P*
Time	3, 53	701.72	< 0.001	328.13	< 0.001
Treatments	4, 55	832.19	< 0.001	333.99	< 0.001
Time*Treatments	4, 55	282.63	< 0.001	99.49	< 0.001

Essential oil compounds from 'thyme'

High levels of suppression of *B. tabaci* populations have been reported previously for the essential oil of thyme (Kim et al. 2011). In this study, thymol (36.18%) was the main constituent of *T. pulegioides* essential oil, followed by carvacrol (13.43%), *p*-Cymene (10.85%) and thymol methyl ether (7.45%). The dominance of thymol and carvacrol chemotypes in the essential oil of thyme is not unusual (Mockute and Bernotiene 2001; Sárosi et al. 2011). In fact, thymol occurs widely in the essential oils of many species within the family Lamiaceae, such as species of *Thymus*, *Monarda* and *Origanum* (Tabanca et al. 2013). Previous studies have revealed that thymol has larvicide and fumigant activity against insect pests as diverse as rice weevils *Sitophilus oryzae*, larvae of the western corn rootworm moth, *Diabrotica virgifera*, and adult house flies, *Musca domestica* L and mosquitoes, *Culex pipiens* (Lee et al. 1997; Rozman et al. 2007; Traboulsi et al. 2002). Suppressive effects are related to the capacity for thymol to disrupt GABAergic pathways and inhibit the nervous systems of pests and their locomotory behavior (Oliveira et al. 2018; Priestley et al. 2003). Our results also confirmed that thymol had a significant repellent effect against *B. tabaci* MED/Q. Similarly, *p*-Cymene was reported to be effective repellents against *B. tabaci* MED/Q (Bleeker et al. 2009; Fang et al. 2013; Li et al. 2016; Shi et al. 2018). Our analysis here shows that *p*-Cymene also has repellent properties, suggesting that some different isomers of the same compound may have a similar role in repelling pests. Lastly, we report significant repellent activity of thymol methyl ether against *B. tabaci* MED/Q (which is a major constituent of the chemotype of *T. pulegioides* essential oil used in this study), which has not previously been recorded, to our knowledge.

Surprisingly, one of the main constituents of the essential oil of thyme, carvacrol, had no significant repellent effects on *B. tabaci* MED/Q, even at the highest concentrations tested. In previous studies, carvacrol has shown significant antifungal, larvicidal, phytotoxic and insecticidal properties against a wide range of organisms, such as mosquitoes, microorganisms and rice weevils (Benelli et al. 2019b; Caglar et al. 2007; Kordali et al. 2008; Petrović et al. 2019; Sökmen et al. 2004; Tabanca et al. 2013; Traboulsi et al. 2002). For *B. tabaci* more specifically, a 24-h fumigant exposure with carvacrol had lower toxicity (LC_{50}, 0.56 μg/cm^3) than thymol (LC_{50}, 0.35 μg/cm^3) or dichlorvos (LC_{50}, 0.20 μg/cm^3) against *B. tabaci*

MED/Q (Chae et al. 2014). This indicates that *B. tabaci* MED/Q may be more tolerant of carvacrol than other volatiles.

Essential oil compounds from 'wormwood'

The essential oil composition of *A. absinthium* has been extensively studied, and at least ten different chemotypes have been recognized previously (Sharopov et al. 2012). In this study, the major constituents of (−)-β-Pinene (27.88%) and linalool (23.41%) were markedly different from those reported for wormwood in European, Middle Eastern or other Asian locations, indicating that this is a new chemotype of wormwood that has not previously been studied (Orav et al. 2006; Sharopov et al. 2012). Almost certainly there are numerous additional chemotypes of *A. absinthium* that remain to be discovered. The observed differences in the constituents of *A. absinthium* essential oils across geographic regions may be due to genetic factors, environmental conditions such as temperature, rainfall, altitude, solar radiation or soil chemistry (amongst others), and even the phenological stage and time of harvesting of plant tissues. Here, (−)-β-Pinene had the greatest repellent action against *B. tabaci* MED/Q, while linalool had a weak effect, even at the highest concentration tested (100 μL). Previous research has found that structural isomers of α-Pinene did not show significant repellent effect against *B. tabaci* MED/Q (Du et al. 2016; Zhao et al. 2012), but no previous studies have tested the effects of β-pinene on whiteflies. Moreover, in other insect species, (−)-β-pinene exhibited only a mild repellent effect against the German cockroach *Blattella germanica* L., whereas some synthetic derivatives of β-pinene possessed greater repellency (Liao et al. 2017). Here, our new findings indicate that (−)-β-pinene is part of a novel suite of repellent chemicals from wormwood that could be used against *B. tabaci* MED/Q, with the appropriate structural isomers used at higher concentrations.

There was a weak (but not statistically significant) indication in the data that a concentration of 0.01 μg of linalool could have a slight attraction effect on *B. tabaci* MED/Q (62.22%, $P < 0.10$). This is consistent with the effect of linalool on *B. tabaci* MEAM1/ B at low concentrations (also 0.01μL) (Cao et al. 2008), but more evidence would be required to confirm the result in *B. tabaci* MED/Q. Certainly, it is known that different concentrations of odorant molecules can activate different signaling pathways, changing the pattern of olfactory receptor neuron activation and evoking different responses (Kaupp 2010; Leal 2013). Linalool has even been described as the multifunctional floral volatile, to manage floral mutualists and antagonists (Raguso 2016).

Potential for eco-friendly approaches to whitefly control

Aromatic plant oils can be developed into products that are environmentally friendly and ideally suited for use in integrated pest management programs. Here, we have demonstrated that the essential oils of *T. pulegioides* and *A. absinthium* have strong repellent effects and contact toxicity on *B. tabaci* MED/Q, supporting their potential use in whitefly management. Natural products, especially those from plant sources, are potentially less hazardous 'eco-friendly' alternatives to conventional synthetic chemical pesticides that can have high off-target impacts. The effectiveness of this approach, however, will hinge on the degree of variability in chemotype and constituent compounds of essential oil extracts and may not have the desired reliability and repeatability of action that farmers would be looking for. Often, variability in effectiveness of mixtures might stem from unrecognized synergistic effects among major and minor compounds of complex blends of several chemical constituents (Akhtar and Isman 2013; Akhtar et al. 2012; Deletre et al. 2016; Waliwitiya et al. 2009). Identifying the main chemical constituents underpinning the mode of repellent action might instead have greater utility in a crop production setting. Here, we identified four such promising compounds (m-Thymol 36.18%, p-Cymene 10.85%, thymol methyl ether 7.45% and linalool 23.41%) with promising repellent action against *B. tabaci* MED/Q. Of course, there is the potential problem of pest desensitization to continued use of any single compound. It was known that pests will develop resistance more slowly to an insecticide composed by several different compounds than to a single ingredient (Isman 2000). Most plants deploy multiple modes of action in a 'multichemical defense' against a variety of potential herbivores (Raffa 1987). These complex mixtures are likely to be more durable with respect to insects evolving resistance and developing behavioral desensitization (Pavela 2007). Therefore, having a suite of four (or more) compounds with high repellent action will provide a range of potential options for deployment (e.g., rotation of compounds, additive mixtures, or designed synthetic derivatives). However, this would require explicit testing of the most effective strategies.

Although essential oils are stronger volatile in the environment, it will disappear to control insects in a hurry. In recent year, nanoencapsulated essential oils impart development in the field and protect active ingredients of the essential oils from the solar light-induced degradation. (Peres et al. 2020), further as to lay foundations to the exploration of commercial products using essential oils. Although the specific biochemical and behavioral pathways by which the volatile monomer components influence whiteflies remain to be tested, this research has laid a solid foundation for the application of 'push–pull strategies' in the comprehensive prevention and control of whiteflies and has broadened the train of thought on aromatic plant oils in more environmentally friendly pest management.

Author contributions

SW, LS, ND and FZ conceived and designed the study. SL and ZQ conducted the experiments. SL and HL analyzed the data and wrote the manuscript, with input from all authors. All authors read and approved the manuscript.

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Declarations

Conflict of interests The authors declare that they have no competing interests.

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