Research Article

Received: 13 October 2014 Revised: 27 January 2015 Accepted article published: 2 February 2015 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.3987

Naturally occurring bioactive compounds from four repellent essential oils against *Bemisia tabaci* whiteflies

Emilie Deletre,[a*] Fabrice Chandre,[b] Barbara Barkman,[a] Chantal Menut[c] and Thibaud Martin[a,d]

Abstract

BACKGROUND: In tropical countries, netting is an effective sustainable tool for protecting horticultural crops against Lepidoptera, although not against small pests such as *Bemisia tabaci*, while smaller mesh netting can be used in temperate regions. A solution is to combine a net with a repellent. Previously we identified repellent essential oils: lemongrass (*Cymbopogon citratus*), cinnamon (*Cinnamomum zeylanicum*), cumin (*Cuminum cyminum*) and citronella (*Cymbopogon winternarius*). The present study was designed to identify the active compounds of these essential oils, characterise their biological activity and examine their potential for coating nets. We investigated the efficiency and toxicity of nets dipped in different solutions. We then studied the repellent effect with an olfactometer and the irritant effect by videotracking.

RESULTS: Geraniol and citronellol were the most promising net coatings owing to their repellent effect. The repellency, irritancy or toxicity varied with the product and concentration, and these features were independent, indicating that the repellent and the irritant/toxic mechanisms were not the same. The combined effects of these different compounds account for the bioactivity of the mixture, suggesting interactions between the compounds.

CONCLUSION: This new sustainable strategy for protecting vegetable crops against whiteflies is discussed, in addition to the use of companion plants that could produce such bioactive compounds.

Keywords: behaviour; repellence; irritation; toxicity; DEET; permethrin

1 INTRODUCTION

The *Bemisia tabaci* whitefly is a serious pest of many field and greenhouse crops in tropical and temperate regions, particularly owing to virus transmission.[1–5] At present, cultivating tomato crops is a real challenge regarding whitefly prevention and control.[2] Whiteflies are hard to control by insecticide foliar sprays as they inhabit the underside of leaves.[6,7] Systemic insecticides, such as neonicotinoids, or chemicals acting on insect development, are thus currently used to treat crops in greenhouses and open fields. However, most of the populations tested worldwide have been diagnosed as resistant to chemical insecticides commonly used in agriculture, such as organophosphates, pyrethroids and neonicotinoids.[8–15] Selection in favour of insecticide resistance population occurs rapidly in whiteflies because of their high fecundity, haplodiploid breeding system and short generation time.[8,16,17] Moreover, the way that insecticides are generally used, i.e excessive year-round use in tropical regions, increases the chance of selection of resistant populations. One way to protect plants from pest insects without using pesticides is to create a visual and physical barrier between the insect and the plant with a net.[18] In Beninese fields, insect-proof nets (IPNs) have been shown to provide more effective protection against the diamondback moth (*Plutella xylostella*) and other Lepidoptera species than foliar insecticide sprays.[19] However, in tropical regions with high temperature and humidity levels, the use of fine mesh nets increases the risk of plant pathogen development. A combination between a large-mesh screen and a repellent or irritant product could be a solution. New compounds now have to be found because of pest resistance to pyrethroids and the low repellent and toxic effects of these chemicals on whiteflies.[20]

Plant-based essential oils appear to be promising as insect repellents/irritants.[21–28] Essential oils are blends of up to several tens of different molecules, two or three of which are usually responsible for their biological activities such as repellence, irritancy or toxicity.[29] Terpenoids, such as citronellal, myrcene, geraniol, citral, limonene, pinene, citronellol and linalool, are the most important chemical group to consider in terms of insect

* *Correspondence to: Emilie Deletre, UR Hortsys, Cirad, Campus de Baillarguet, 34980 Montferrier, France. E-mail: emilie.deletre@cirad.fr*

a *UR Hortsys, Cirad, Campus de Baillarguet, Montferrier, France*

b *UMR MiVEGEC, IRD-CNRS-UM1-UM2, Monferrier, France*

c *Institut des Biomolécules Max Mousseron, Faculté de Pharmacie, Montpellier, France*

d *Plant Health Department, ICIPE, Nairobi, Kenya*

repellency.[30] In a previous study, we identified four highly repellent essential oils among 20 essential oils: *Cymbopogon citratus*, *Cymbopogon winterianus*, *Cuminum cyminum* and *Cinnamomum zeylanicum*. IPNs treated with 1% (w/w or v/v) of these essential oils showed the following *B. tabaci* net-crossing rates: 42.9, 54.3, 72.4 and 13.8% respectively (Deletre E and Mallent M *et al.* accepted for publication).[31] A toxic effect was observed after 4 h of exposure: 96.3% mortality for cinnamon oil, 64.7% for citronella oil, 61.0% for lemongrass oil and 30.0% for cumin oil. However, although these essential oils were shown to be repellent and toxic, their active compounds are still unidentified, and they could be one or several major compounds.

The objectives of this study were: (1) to identify and quantify the compounds from these essential oils; (2) to evaluate, by means of behavioural assays, the bioactive effects of the major compounds, either alone or combined, to shed light on the potential efficacy of essential oils or their active compounds as an appropriate supplement to the physical barrier of IPNs. We tested two hypotheses: (1) the biological effects of essential oils are only due to the effect of the major compound; (2) the biological effects of essential oils are the result of a synergic/additive effect of many compounds.

2 MATERIALS AND METHODS

2.1 Insects

B. tabaci biotype Q (MPL strain) whiteflies were reared on tomato plants (*Solanum lycopersicum* L.) in a climatic room at 27 ± 1 °C and 50 ± 10% relative humidity with a 12:12 h light:dark photoperiod.

2.2 Chemicals

Studies were carried out with four plant essential oils – lemongrass (leaves), *Cymbopogon citratus* (IBMM, France), citronella (bark), *Cymbopogon winterianus* (Nactis, France, lot 40018500), cumin (seeds), *Cuminum cyminum* (Ipra, France, lot 902560), and cinnamon (bark), *Cinnamomum zeylanicum* (Nactis, France) – and with 13 chemical standards (Sigma Aldrich, St Louis, MO): citral (95% purity), citronellal (≥95% purity), geraniol (98% purity), citronellol (≥95% purity), (*S*)-(−)-limonene (96% purity), geranyl acetate (98% purity), cuminaldehyde (98% purity), (−)-β-pinene (99% purity), γ-terpinene (≥97% purity), *p*-cymene (99% purity), (*E*)-cinnamaldehyde (99% purity), 2-methoxycinnamaldehyde (98% purity) and cinnamyl acetate (99% purity). DEET and permethrin were used as positive control. Indeed, DEET is one of the most famous insect repellents, and permethrin is a toxic irritant pyrethroid that is used against most insects. DEET, permethrin and the four mixtures of major essential oil compounds available on the market – lemongrass mixture (citral, geraniol and geranyl acetate), citronella mixture (citronellal, geraniol, citronellol, limonene and geranyl acetate), cumin mixture (cuminaldehyde, β-pinene, γ-terpinene and *p*-cymene) and cinnamon mixture [(*E*)-cinnamaldehyde, 2-methoxycinnamaldehyde and cinnamyl acetate] – were diluted at 0.1 and 1% (v/v for liquid compounds or w/w for powdered compounds) in ethanol. Each mixture was prepared by diluting the major compounds in ethanol in a ratio based on their respective proportions in the essential oils. All major compounds were tested at the relative concentration at which they are found in the essential oils at 1 and 0.1%[32] (Table 1). For instance, citronellal represents 34.7% of citronella essential oil. Citronella oil was efficient at 1%, so citronellal was tested at C2 = 0.35%, i.e. 0.25 mg mL^{-1} ($d_{ethanol}$: 0.789 g mL^{-1}, $d_{citronellal}$: 0.885 g mL^{-1}), and at tenfold less C1 = 0.035%, i.e. 0.025 mg mL^{-1}. Each mixture was created with the compounds in their respective proportions in the essential oils. By doing this, the quantity of a compound was the same when the essential oil, the mixture and the compound alone were tested. Each assay was preceded by a negative control in which only ethanol was tested.

2.3 Gas chromatography analysis

The four essential oils (citronella, cinnamon, cumin and lemongrass) were analysed on a CP-3380 gas chromatograph (Varian, Palo Alto, CA) equipped with a flame ionisation detector (FID) at 220 °C and using an apolar HP_5 J&W Agilent (5% phenyl–95% methylpolysiloxane) capillary column (30 m × 0.25 mm, film thickness 0.25 µm; Agilent, Santa Clara, CA). Injector and detector temperatures were set at 220 and 250 °C respectively. The oven temperature was maintained at 60 °C for 1 min and programmed at 3 °C min^{-1} to 220 °C. N_2 was the carrier gas, at a 0.8 mL min^{-1} flow rate. A 1 µL solution (10% essential oil in ethyl ether) was manually injected. A mixture of alkanes (C9–C22) was injected to calculate the retention index: RI = [TR(X) − TR(*n*)]/[TR(*n* + 1) − TR(*n*)]*100 + 100**n*, where TR(X) is the retention time of the studied product, TR(*n*) is the retention time of the alkane with *n* carbons eluted before X, and TR(*n* + 1) is the retention time of the alkane of *n* + 1 carbons eluted after X. The percentage composition of the essential oil was computed by the normalisation method from GC/FID analyses, with the response factors being taken as one for all compounds.

2.4 Coupled gas chromatography–mass spectrometry analysis

GC-MS analyses were performed using a Hewlett Packard (Palo Alto, CA) 5890 II gas chromatograph interfaced with a quadrupole detector (model 5972) and equipped with an HP-5 MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm). Helium was the carrier gas, at a 0.6 mL min^{-1} flow rate. Injector and MS transfer line temperatures were set at 220 and 250 °C respectively. The oven programme temperature was the same as that used in the GC-FID analysis. Diluted samples (10:100 in CH_2Cl_2, v/v) of 1 µL were injected manually and in split mode (1:100). MS was performed in EI mode at 70 eV, in the *m/z* 35–300 range; electron multiplier 1460 eV; scan rate 2.96 scans s^{-1}. The constituents were identified on the basis of comparisons of their relative retention indices and mass spectra with those of standards (for the main components), those found in the literature[33] and those supplemented by the NBS75K database and Wiley 7th NIST 98 EPA/NIH Mass Spectral Library Upgrade (provided by Hewlett Packard with the GC/MS control and data processing software).

2.5 Bioassays

Detailed descriptions of the apparatus, assay protocol and data analysis procedures were previously published (Deletre E and Mallent M *et al.* accepted for publication).[31] Bioassays were conducted between 10 a.m. and 6 p.m. at 24 ± 1 °C and 50 ± 10% RH. For each product, all assays were performed the same day, with only one product tested per day from the lowest to the highest concentration. The apparatuses were washed with a highly detergent and decontaminating solution (TFD4; Franklab, Montigny-le-Bretonneux, France) at 20% (v/v).

2.5.1 Toxicity bioassays

Two transparent plastic tubes (length 10 cm, diameter 5 cm; Dominique Dutscher SAS®, Brumath, France) were separated

Table 1. Ratios and quantities of citronella, cumin, cinnamon and lemongrass essential oil compounds

Essential oil	Composition (%)[a]		Quantity tested (mg mL^{-1})[b] C1	C2
Citronella *Cymbopogon winterianus*	34.7%	citronellal	0.291	2.91
	22.5%	geraniol	0.205	2.05
	12.0%	citronellol	0.102	1.02
	3.5%	geranyl acetate	0.037	0.37
	3.3%	limonene	0.025	0.25
	76.0%	**subtotal (mixture)**		
	4.2%	elemol	NT[c]	NT
	2.9%	citronellyl acetate	NT	NT
	2.5%	β-elemene	NT	NT
	2.2%	δ-cadinene	NT	NT
	0.9%	linalol	NT	NT
	0.8%	eugenol	NT	NT
	89.5%	**total**		
Cumin *Cuminum cyminum*	30.1%	cuminaldehyde	0.293	2.93
	12.2%	β-pinene	0.087	0.87
	11.6%	γ-terpinene	0.085	0.85
	9.7%	*p*-cymene	0.086	0.86
	63.6%	**subtotal (mixture)**		
	16.6%	*p*-mentha-1,3-dien-7-al	NT	NT
	8.8%	*p*-mentha-1,4-dien-7-al	NT	NT
	0.6%	α-pinene	NT	NT
	0.4%	myrcene	NT	NT
	0.4%	limonene	NT	NT
	90.4%	**total**		
Cinnamon, *Cinnamomum zeylanicum*	78.5%	(*E*)-cinnamaldehyde	0.840	8.40
	9.6%	2-methoxycinnamaldehyde	0.090	0.90
	3.1%	cinnamyl acetate	0.032	0.32
	91.2%	**subtotal (mixture)**		
	1.1%	benzaldehyde	NT	NT
	0.9%	coumarine	NT	NT
	0.7%	phenyl ethyl alcohol	NT	NT
	0.4%	(*Z*)-cinnamaldehyde	NT	NT
	94.3%	**total**		
Lemongrass *Cymbopogon citratus*	74.1	citral	0.714	7.14
	4.5	geraniol	NT	NT
	3.9	geranyl acetate	0.037	0.37
	82.5	**subtotal (mixture)**		
	1.9	limonene	NT	NT
	1.8	β-caryophyllene	NT	NT
	0.7	linalool	NT	NT
	1.5	borneol	NT	NT
	0.6	nerol	NT	NT
	89.0	**total**		

[a] The percentage composition of the essential oil was computed by the normalisation method from GC/FID analyses, with response factors taken as one for all compounds. The composition of the four essential oils was identified by gas chromatography and mass spectrometry.
[b] The used quantities are expressed in mg mL^{-1} of solution in which the nets were dipped.
[c] NT = not tested.

by a polyethylene net with 40 holes cm^{-2} and a mesh size of about 0.9 mm (A to Z Textile Mills Ltd, Arusha, Tanzania). A 36 cm^2 net was dipped for 10 s in the different solutions and dried for 15 min under an extractor hood. Black cardboard wrapped in an aluminium sheet covered one tube to ensure darkness (dark tube). The other tube (uncovered) was called the light tube. The apparatus was oriented horizontally under a light source in a climatic chamber (27 ± 1 °C, 50 ± 10% RH). After 1 min in the freezer (−20 °C), between 100 and 200 *B. tabaci* adults (mixed sex and age) were placed in the dark tube. For each product, each concentration was replicated 6 times simultaneously. The number of whiteflies and their status (alive or dead) were recorded for each tube after 4 h to establish the whitefly net-crossing rate and the mortality.[20] Then, dead and live whiteflies that had crossed

through the net after the 4 h were separately placed on tomato leaves (*Solanum lycopersicum* L.) and placed on agar gel (1%) in a petri dish. They were preserved in the climatic chamber (27 ± 1 °C, 50 ± 10% RH), and then the mortality rate was determined after 24 h.

2.5.2 *Irritancy bioassays*

For each compound, the surface of a 12 × 15 cm section of black paper was treated with 2 mL of solution or 97% ethanol for the control before drying.[32] The paper was always prepared on the same day it was used, and between trials the paper was stored at −20 °C. Only one product was tested per day to avoid contaminations. The irritant test was carried out with citronellal, geranyl acetate, cuminaldehyde, 2-methoxycinnamaldehyde and citral at 1% and with cinnamaldehyde and cinnamyl acetate at 0.5%, as well as with the positive controls DEET and permethrin at 1%. The choice test was conducted on a 16 cm^2 area (arena), where half of the surface was treated paper (treated zone) and the other half was control paper (control zone). The areas were delineated with a 2 mm thick cardboard border and a plexiglas cover to prevent whiteflies from escaping during the experiment and to force them to walk on the paper and not fly. *B. tabaci* were placed at the centre of the arena, one per trial. Their activity – time spent moving, average speed when moving, distance moved and time spent in each zone – was monitored over a 10 min period. The experiment was repeated 30 times with different individuals, and after five recordings the arena was replaced with another one and the orientation changed. The apparatus for the no-choice test consisted of a similar set-up but with two 9 cm^2 arenas – one arena was treated and the other was the control (97% ethanol). *B. tabaci* whiteflies were placed in the arena. Their activity – time spent moving, average speed when moving and distance moved – was monitored over a 10 min period. The experiment was repeated 20 times (10 times for the treated arena and 10 times for the control arena per test compound) with different individuals, and after five recordings the arena was replaced with another one. The monitoring was done using a video camera (25 frames s^{-1}) fixed above the arena, and the images were analysed using the Ethovision video observation system (Noldus Information Technology, Wageningen, The Netherlands), which is designed to automate animal behaviour observations.[34]

2.5.3 *Repellence bioassays*

A still-air olfactometer was oriented vertically under a light source (two white light tubes, 30 cm, 8 W) to assess the repellent effect of the essential oils.[7] A glass cylinder (length 30 cm, diameter 3 cm; Legallais Society®, Montferrier-sur-Lez, France) was closed at the top with a very fine mesh net that whiteflies could not pass through, along with a treated filter paper and a glass stopper, in this order, and the bottom was closed with a glass stopper pierced with a cylinder (length 10 cm, diameter 0.5 cm) (Deletre E and Mallent M *et al.* accepted for publication).[31] A quantity of 40 µL of each compound or ethanol (control) was placed on the 4 cm^2 piece of non-woven fabric filter paper. The filter papers were dried for 5 min under an extractor hood. The concentrations of each compound were tested in different trials, and four replications per concentration were carried out simultaneously with four controls under an extractor hood. The cylinder was divided into three parts: the top part from 0 to 2 cm to the top of the cylinder, the bottom part from 0 to 10 cm to the bottom of the cylinder and the middle part between these two parts (Deletre E and Mallent M *et al.* accepted for publication).[31] After 1 min in the freezer, 10–20 *B. tabaci* adults (mixed sex and age) were placed at the bottom of the cylinder. After 1 h, the number of whiteflies was recorded in each part, along with the number of dead individuals.

2.6 Data analysis

We used the same method to analyse the proportion of dead whiteflies in the toxicity assays and the net-crossing rate in the irritancy assays. The data analysis was carried out using the R 2.12.2 software package.[35] To compare the proportions of escaped or dead whiteflies in the control and treatment assays, we used Fisher's exact test corrected according to Bonferroni using the Holm's sequential method.[36] Then the proportions of escaped or dead whiteflies were corrected on the basis of the control assay values using the Sun–Shepard formula.[37] For all products and concentrations, these corrected proportions were used to perform a principal component analysis (PCA). Then a hierarchical ascendant classification (HAC) based on Ward's algorithm was used to group the compounds on the basis of the similarity of their effects using PCA axis coordinates. This process yielded a binary segmentation tree, reflecting the hierarchy of similarities between responses to compounds. The optimal number of classes in the tree was determined by the decrease in the interclass variance. For the repellent bioassays, whitefly distributions within the olfactometer cylinders were compared between control and treatment cylinders using Fisher's exact test. The choice irritancy assay data were analysed using a paired *t*-test or a Wilcoxon test in the case of non-normally distributed data. For the no-choice irritancy assay, the data were analysed using an unpaired *t*-test or a Wilcoxon test in the case of non-normally distributed data.

3 RESULTS

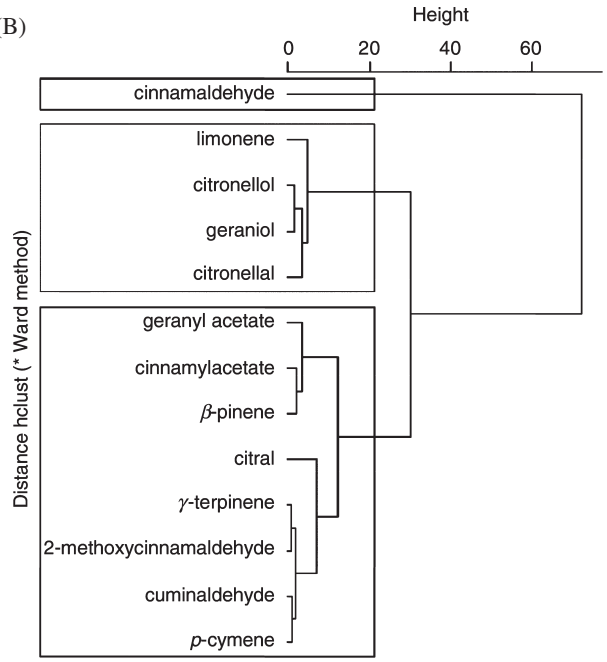
3.1 Toxicity bioassays

The whitefly net-crossing rate was significantly reduced by DEET, permethrin, the four compound mixtures and the following pure compounds: cinnamaldehyde, cinnamyl acetate, β-pinene, γ-terpinene, citronellal, geraniol, citronellol, limonene and citral (Fig. 1). After 4 h, the percentage mortality had significantly increased in the case of cinnamaldehyde, cinnamyl acetate, γ-terpinene, citronellal, geraniol, citronellol, limonene and citral (Fig. 2). After 24 h, the mortality significantly increased for the cumin and lemongrass mixtures and the cinnamyl acetate, cuminaldehyde, *p*-cymene, geraniol and citronellol compounds (Fig. 3). According to the HAC, the most promising compounds to prevent *B. tabaci* from crossing through the net are cinnamaldehyde, citronellal and limonene. The effect of the cinnamon mixture appeared to be due to cinnamaldehyde, i.e. at high concentration all whiteflies were dead after 4 h, even though some whiteflies succeeded in passing through the treated net before dying. At high concentration, the effect of the cumin mixture limited the whitefly net-crossing rate by killing them, but when these pests did succeed in escaping, their mortality was low after 24 h. Although cuminaldehyde showed a toxic effect, it could not explain the effect observed with the mixture, suggesting synergism between the compounds. At high concentration, the citronella mixture, like the cumin mixture, reduced the whitefly net-crossing rate by killing them, but when they succeeded in escaping, their mortality was low after 24 h. Citronellol, citronellal, geraniol and limonene also showed toxic effects and decreased the whitefly net-crossing rate, but to a lesser extent than the citronella mixture, suggesting a synergic or additive effect between these four

(A)

Compound	C1	C2
DEET	**64.5*** **(60.2–68.8)**	**47.3 (44.0–50.6)**
Permethrin	**56.7 (53.4–60.0)**	**1.6 (1.3–1.9)**
Cinnamon[1]	**87.5 (85.7–89.3)**	**53.3 (51.5–55.1)**
Cinnamaldehyde	96.2 (94.2–98.2)	**48.9 (47.8–50.0)**
Cinnamyl acetate	**90.1 (87.0–93.2)**	92.1 (89.1–95.1)
2-methoxycinnamaldehyde	95.8 (94.0–97.6)	93.8 (91.4–96.2)
Cumin[2]	**85.9 (83.7–88.1)**	**66.8 (64.0–69.6)**
Cuminaldehyde	96.1 (93.9–98.3)	94.6 (92.4–96.8)
β-pinene	**90.4 (86.8–94.0)**	94.1 (91.3–96.9)
γ-terpinene	95.4 (93.2–97.6)	**93.2 (90.3–96.1)**
ρ-cymene	95.3 (92.7–97.9)	94.8 (92.1–97.5)
Citronella[3]	90.5 (87.2–93.8)	**61.8 (59.7–63.9)**
Citronellal	92.0 (87.7–96.3)	**87.8 (83.0–92.6)**
Geraniol	89.1 (84.0–94.2)	**86.0 (83.0–89.0)**
Citronellol	**90.6 (87.3–93.9)**	**85.9 (82.6–89.2)**
Geranyl acetate	93.0 (89.4–96.6)	92.1 (88.3–95.9)
Limonene	91.0 (86.1–95.9)	**82.8 (78.4–87.2)**
Lemongrass[4]	**84.4 (82.4–86.4)**	**49.2 (46.8–51.6)**
Citral	96.5 (94.7–98.3)	**89.5 (86.7–92.3)**

(B)



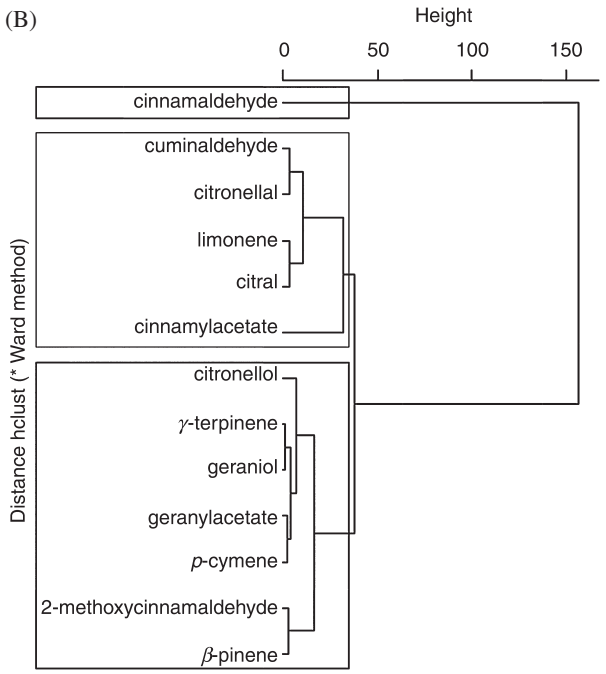
* *P*-values determined with Fisher's exact test, bold values indicate statistical significance.
[1] Cinnamon mixture: 78.51% cinnamaldehyde, 9.65% 2-metoxycinnamaldehyde, 3.15% cinnamylacetate.
[2] Cumin mixture: 30.09% cuminaldehyde, 12.19% β-pinene, 11.59% γ-terpinene, 9.74 *p*-cymene.
[3] Citronella mixture: 34.74% citronellal, 22.50% geraniol, 12.03% citronellol, 3.51% geranyl acetate, 3.34% limonene.
[4] Lemongrass mixture: 74.08% citral, 4.5% geraniol, 3.9% geranyl acetate.

Figure 1. The whitefly net-crossing rate measured after 4 h through a net treated with DEET, permethrin, the major compounds of four essential oils and their mixture at two different concentrations (C1 and C2, mg mL^{-2} of product, see Table 1): (A) corrected proportion escaping using the Sun–Shepard formula (confidence interval calculated by Wald's method) by treatment concentration; (B) dendrogram determined by hierarchical ascendant classification.

(A)

Compound	C1	C2
DEET	**97.6*** **(96–99.2)**	**100.0 (100.0–100.0)**
Permethrin	**94.6 (93.4–95.8)**	**100.0 (100.0–100.0)**
Cinnamon[1]	6.5 (4.8–8.2)	**100.0 (98.2–100.0)**
Cinnamaldehyde	0.5 (0.0–2.0)	**100.0 (100.0–100.0)**
Cinnamyl acetate	**23.9 (20.6–27.2)**	**20.1 (16.6–23.6)**
2-methoxycinnamaldehyde	1.1 (0.5–1.7)	2.1 (0.9–3.3)
Cumin[2]	10.1 (8–12.2)	**57.0 (55.1–58.9)**
Cuminaldehyde	2.9 (1.2–4.6)	**14.7 (12.2–17.2)**
β-pinene	3.0 (1.4–4.6)	0.0 (0.0–1.4)
γ-terpinene	2.6 (1.4–3.8)	**8.5 (6.7–10.3)**
ρ-cymene	3.8 (1.9–5.7)	6.3 (4.3–8.3)
Citronella[3]	0.7 (0.0–2.2)	**65.8 (63.8–67.8)**
Citronellal	1.4 (0.0–4.1)	**11.5 (6.2–16.8)**
Geraniol	3.3 (0.7–5.9)	**8.1 (6.2–10.0)**
Citronellol	**8.1 (6.1–10.1)**	**10.0 (8.0–12.0)**
Geranyl acetate	5.6 (3.9–7.3)	7.1 (5.2–9.0)
Limonene	4.7 (0.6–8.8)	**19.7 (15.3–24.1)**
Lemongrass[4]	**17.6 (16.4–18.8)**	**100.0 (100.0–100.0)**
Citral	1.3 (0.0–2.7)	**19.5 (18.1–20.9)**

(B)



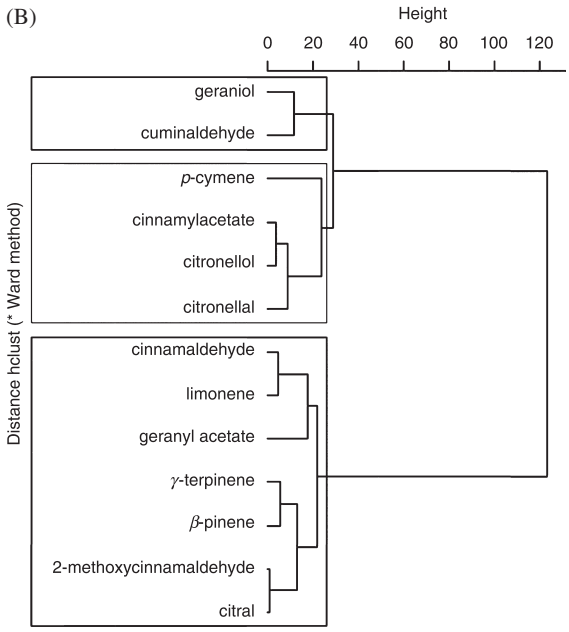
* *P*-values determined with Fisher's exact-test, bold values indicate statistical significance.
[1] Cinnamon mixture: 78.51% cinnamaldehyde, 9.65% 2-metoxycinnamaldehyde, 3.15% cinnamylacetate.
[2] Cumin mixture: 30.09% cuminaldehyde, 12.19% β-pinene, 11.59% γ-terpinene, 9.74 *p*-cymene.
[3] Citronella mixture: 34.74% citronellal, 22.50% geraniol, 12.03% citronellol, 3.51% geranyl acetate, 3.34% limonene.
[4] Lemongrass mixture: 74.08% citral, 4.5% geraniol, 3.9% geranyl acetate.

Figure 2. The whitefly mortality rate measured after 4 h through a net treated with DEET, permethrin, the major compounds of four essential oils and their mixture at two different concentrations (C1 and C2, mg mL^{-2} of product, see Table 1): (A) corrected proportion using the Sun–Shepard formula (confidence interval calculated by Wald's method) by treatment concentration; (B) dendrogram determined by hierarchical ascendant classification.

(A)

Compound	C1		C2	
DEET	0.0*	(0.0–0.6)	0.0	(0.0–0.0)
Permethrin	2.1	(0.0–6.0)	0.0	(0.0–0.0)
Cinnamon[1]	3.3	(0.1–6.5)	0.0	(0.0–0.0)
Cinnamaldehyde	4.0	(0.4–7.6)	0.0	(0.0–0.0)
Cinnamyl acetate	**18.7**	**(14.2–23.2)**	**13.8**	**(9.2–18.4)**
2-methoxycinnamaldehyde	0.0	(0.0–0.0)	0.0	(0.0–0.0)
Cumin[2]	15.0	(9.8–20.2)	**26.7**	**(22.4–31.0)**
Cuminaldehyde	**20.9**	**(15.2–26.6)**	**23.5**	**(19.3–27.7)**
β-pinene	1.3	(0.0–7.3)	1.5	(0.0–7.2)
γ-terpinene	3.6	(0.0–8.1)	6.2	(0.6–11.8)
ρ-cymene	2.5	(0.0–8.3)	**18.5**	**(11.9–25.1)**
Citronella[3]	2.4	(0.0–6.5)	9.9	(3.8–16.0)
Citronellal	**21.3**	**(11.9–30.7)**	8.7	(0.0–17.7)
Geraniol	**28.3**	**(19.9–36.7)**	**32.1**	**(25.7–38.5)**
Citronellol	**17.2**	**(10.6–23.8)**	**17.1**	**(10.8–23.4)**
Geranyl acetate	0.0	(0.0–8.7)	0.0	(0.0–7.3)
Limonene	8.6	(0.5–16.7)	0.0	(0.0–7.5)
Lemongrass[4]	**24.8**	**(18.8–30.8)**	0.0	(0.0–0.0)
Citral	0.0	(0.0–2.8)	0.0	(0.0–0.0)

(B)



* *P*-values determined with Fisher's exact-test, bold values indicate statistical significance.
[1] Cinnamon mixture: 78.51% cinnamaldehyde, 9.65% 2-metoxycinnamaldehyde, 3.15% cinnamylacetate.
[2] Cumin mixture: 30.09% cuminaldehyde, 12.19% β-pinene, 11.59% γ-terpinene, 9.74 *p*-cymene.
[3] Citronella mixture: 34.74% citronellal, 22.50% geraniol, 12.03% citronellol, 3.51% geranyl acetate, 3.34% limonene.
[4] Lemongrass mixture: 74.08% citral, 4.5% geraniol, 3.9% geranyl acetate.

Figure 3. The whitefly mortality rate measured after 24 h in the presence of a net treated with DEET, permethrin, the major compounds of four essential oils and their mixture at two different concentrations (C1 and C2, mg mL^{-2} of product, see Table 1): (A) corrected proportion using the Sun–Shepard formula (confidence interval calculated by Wald's method) by treatment concentration; (B) dendrogram determined by hierarchical ascendant classification.

Table 2. Results of the Ethovision choice irritancy test. For all tested compounds the average results of all replicates and *P*-values (paired *t*-test or Wilcoxon's test) are given for the distance moved, time spent moving (mobility), average velocity and time spent in each zone

		Total distance moved (mm)		Mobility (%)		Average velocity ($mm.s^{-1}$)		Time spent in zones (s)	
		Treated	Control	Treated	Control	Treated	Control	Treated	Control
2-Methoxycinnamaldehyde	Average	202.69	202.94	27.03	24.13	2.81	2.79	251.47	348.59
	P-value	0.643[b]		0.527[a]		0.7685[b]		0.176[a]	
Cinnamaldehyde	Average	146.10	127.81	17.64	18.87	2.80	2.76	358.68	241.40
	P-value	0.459[b]		0.782[b]		0.263[b]		0.393[a]	
Cinnamyl acetate	Average	200.37	226.17	24.80	32.61	2.71	2.82	176.70	305.37
	P-value	0.581[a]		0.024[b]		0.73[b]		0.832[a]	
Citral	Average	343.40	316.05	37.86	38.41	3.01	2.97	300.35	299.73
	P-value	0.564[a]		0.833[b]		0.29[b]		0.991[a]	
Citronellal	Average	298.55	307.96	34.21	35.44	1.07	1.14	312.74	287.34
	P-value	0.968[b]		0.503[b]		0.428[b]		0.627[a]	
Cuminaldehyde	Average	216.57	240.72	31.85	27.14	2.78	2.71	254.92	344.60
	P-value	0.395[a]		0.173[b]		0.062[a]		0.117[a]	
Geranyl acetate	Average	318.19	359.67	35.89	40.77	2.96	2.96	314.06	286.02
	P-value	0.385[a]		0.005[b]		0.971[a]		0.543[a]	
DEET	Average	256.63	337.40	35.24	30.05	3.19	3.19	247.04	353.05
	P-value	0.269[b]		0.043[b]		0.515[b]		0.157[a]	
Permethrin	Average	153.84	154.92	15.80	19.55	2.63	2.48	351.15	248.93
	P-value	0.570[b]		0.184[b]		0.39[b]		0.205[b]	

[a] Tested with a paired *t*-test.
[b] Tested with Wilcoxon's test.

products. With the lemongrass mixture, all whiteflies were dead after 4 h, although some of them succeeded in crossing through the treated net before dying. Citral alone could not explain the effect of the lemongrass mixture. Thus, geraniol and limonene could play a role in the toxic effect of the lemongrass mixture. The two positive controls, i.e. DEET and permethrin, were very toxic, and the number of whiteflies that passed through the treated net was relatively low.

3.2 Irritancy bioassays

In the choice assay, the time spent in the treated zone and the time spent in the control zone did not differ for any of

Table 3. Results of the Ethovision no-choice irritancy test. For all tested compounds the average results of all replicates and *P*-values (unpaired *t*-test or Wilcoxon's test) are given for the distance moved and time spent

		Total distance moved (mm)		Mobility (%)		Average velocity (mm s^{-1})	
Compound		Treated	Control	Treated	Control	Treated	Control
2-Methoxycinnamaldehyde	Average	472.04	450.43	27.50	24.18	2.84	2.97
	P-value	0.870[a]		0.84[b]		0.44[a]	
(*E*)-Cinnamaldehyde	Average	571.44	478.43	28.51	24.64	3.18	3.27
	P-value	0.50[a]		0.50[b]		0.97[b]	
Cinnamyl acetate	Average	273.80	290.20	0.24	0.26	1.86	1.32
	P-value	0.83[a]		0.72[b]		0.21[a]	
Citral	Average	152.66	345.06	0.14	0.30	1.52	1.87
	P-value	0.02[a]		0.04[b]		0.15[b]	
Citronellal	Average	253.38	393.89	0.22	0.34	0.42	0.66
	P-value	0.11[a]		0.14[b]		0.11[a]	
Cuminaldehyde	Average	249.67	179.26	0.22	0.16	1.58	1.82
	P-value	0.21[a]		0.19[b]		0.41[b]	
Geranyl acetate	Average	246.34	364.05	0.23	0.32	1.91	1.63
	P-value	0.11[a]		0.08[b]		0.39[b]	
DEET	Average	186.10	195.79	0.16	0.17	1.92	1.34
	P-value	0.86[a]		0.90[b]		0.31[b]	
Permethrin	Average	435.33	446.80	24.83	24.36	2.90	3.02
	P-value	0.91[a]		0.97[b]		0.25[a]	

[a] Tested with an unpaired *t*-test.
[b] Tested with Wilcoxon's test.

the tested compounds (Table 2). There was no movement away from the treated zone. The whitefly activity, i.e. mobility, velocity and distance moved, did not differ between the treated areas and the control, except for areas treated with cinnamyl acetate and geranyl acetate, where whiteflies were significantly less mobile than on the control, and for DEET, where they were more mobile. In the no-choice assay, there was a difference in activity between the treated and the non-treated areas only for citral, where there was less distance covered and mobility than on the treated arena (Table 3). These bioassays showed that it was not because the product was irritant that *B. tabaci* did not cross though the net.

3.3 Repellence bioassays

A compound is repellent when the vapour toxicity is low and most of the insects were in the bottom portion of the olfactometer. DEET was repellent at 0.1 and 1%, whereas permethrin was not repellent at 1% (Table 4). The cinnamon mixture was repellent at 0.1% and caused a high vapour mortality at 1%, and among the compounds only cinnamaldehyde was repellent irrespective of dose, with 30% mortality. The cumin mixture was repellent at 0.1 and 1%, and among the pure compounds only cuminaldehyde was repellent at 0.3%. The citronella mixture was repellent at 0.1 and 1%, with high vapour toxicity, and among the pure compounds, geraniol, citronellol and geranyl acetate were repellent at their highest concentration, and citronellal showed high vapour toxicity at 0.34%. The lemongrass mixture was repellent at 0.1% and caused high vapour mortality at 1%, and among the pure compounds only geraniol was repellent at the highest concentration and citral caused high vapour toxicity at 0.8%. At a lower dose, the citral was not toxic, and the majority of whiteflies were in the upper portion of the olfactometer, indicating that this compound was not repellent.

4 DISCUSSION

We showed that cinnamaldehyde, cuminaldehyde, geraniol, citronellol and geranyl acetate were repellent compounds (Table 5), and that this effect depended on the concentration used. We have already shown a repellent effect of these compounds in another insect species, *Anopheles gambiae*.[32] In many studies, particularly with mosquitoes, geraniol and citronellol showed repellent properties.[23,25,38,39] A recent study showed the deterrence and toxicity effects of citronellol and geraniol on *Bemisia tabaci*.[40] Moreover, geraniol and citronellol from the essential oil *Dianthus caryophyllum* also showed repellent effects against *Ixodes ricinus* ticks.[41] A cinnamon mixture, citronella mixture, lemongrass mixture, citronellal and citral showed high vapour toxicity, and cinnamaldehyde, geraniol, citronellol and geranyl acetate also showed vapour toxicity, but lower. The essential oils of *Cymbopogon nardus* [citronellal (33.8%), geraniol (21.6%), citronellol (9.2%), geranyl acetate (3.2%)] and *Cinnamomum verum* [cinnamaldehyde (90%)] also showed vapour toxicity against the bean weevil *Acanthoscelides obtectus*, along with the essential oil of *Cuminum cyminum* [cuminaldehyde (42.5%), pinene (11.8%), terpinene (11.4%)], which had no vapour toxicity against *Bemisia tabaci*.[42] The four mixtures and cinnamaldehyde showed high toxicity, and cinnamyl acetate, cuminaldehyde, γ-terpinene, citronellal, citronellol, geraniol, limonene and citral also showed a toxic effect, but lower. Huang and Ho[43] also highlighted the toxicity and antifeedant activities of cinnamaldehyde against the grain storage insects *Tribolium castaneum* and *Sitophilus zeamais*. We noted that aldehydes and alcohols were the active components of the essential oils. Many articles have been published on essential oil effects, but few have focused on the behavioural effects of their compounds, thus illustrating the lack of knowledge on the action mechanisms of essential oils. As specialised odourant-binding proteins in the sensilla of insects respond to volatile monoterpenes,

Table 4. Distribution and mortality rate of *Bemisia tabaci* adults (confidence interval calculated by the Wald method) among three parts of a vertical olfactometer exposed to DEET, permethrin, natural compounds and four mixtures at different concentrations

Product	Concentration (%) (mg L^{-1})	*P*-value[a]	Control				Treated			
			Top (%) (CI)	Middle (%) (CI)	Bottom (%) (CI)	Dead (%) (CI)	Top (%) (CI)	Middle (%) (CI)	Bottom (%) (CI)	Dead (%) (CI)
DEET	0.1 (0.998)	<0.001	92.9 (87.5–98.3)	4.7 (0.2–9.2)	1.2 (0.0–3.5)	1.2 (0.0–3.5)	0.0 (0.0–0.0)	21.8 (12.6–31)	78.2 (69–87.4)	0.0 (0.0–0.0)
	1 (9.98)	<0.001	87.9 (81.5–94.3)	8.1 (2.7–13.5)	2.0 (−0.8–4.8)	2.0 (0.0–4.8)	5.1 (0.8–9.4)	28.3 (19.4–37.2)	66.7 (57.4–76.0)	0.0 (0.0–0.0)
Permethrin	1 (11.9)	0.055	86.7 (80.4–93.0)	10.6 (4.9–16.3)	0.9 (0.0–2.6)	1.8 (0.0–4.2)	73.5 (66.1–80.9)	21.3 (14.4–28.2)	5.1 (1.4–8.8)	0.0 (0.0–0.0)
Cinnamon[b]	0.1	<0.001	84.2 (76.0–92.4)	13.2 (5.6–20.8)	1.3 (0.0–3.9)	1.3 (0.0–3.9)	0.0 (0.0–0.0)	6.5 (1.0–12.0)	76.6 (67.1–86.1)	16.9 (8.5–25.3)
	1	<0.001	82.5 (74.2–90.8)	12.5 (5.3–19.7)	3.8 (0.0–8.0)	1.3 (0.0–3.7)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	7.2 (1.6–12.8)	92.8 (87.2–98.4)
Cinnamaldehyde	0.008 (0.08)	<0.001	73.2 (63.9–82.5)	15.9 (6.8–25.0)	9.8 (1.2–18.4)	1.2 (0.0–3.5)	0.0 (0.0–0.0)	7.3 (1.2–13.4)	59.8 (47.0–72.6)	32.9 (20.6–45.2)
	0.08 (0.84)	<0.001	73.5 (64.0–83.0)	18.1 (9.8–26.4)	7.2 (1.6–12.8)	1.2 (0.0–3.5)	0.0 (0.0–0.0)	4.3 (0.0–10.2)	65.2 (51.4–79.0)	30.4 (17.1–43.7)
	0.8 (8.40)	<0.001	76.1 (66.2–86.0)	11.3 (3.9–18.7)	12.7 (5.0–20.4)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	1.8 (0.0–5.3)	66.1 (53.7–78.5)	32.1 (19.9–44.3)
Cinnamyl acetate	0.003 (0.03)	0.145	73.4 (62.6–84.2)	15.6 (6.7–24.5)	4.7 (0.0–9.9)	6.3 (0.4–12.2)	88.9 (80.5–97.3)	9.3 (1.6–17.0)	0.0 (0.0–0.0)	1.9 (0.0–5.5)
	0.03 (0.32)	0.986	67.3 (54.9–79.7)	14.5 (5.2–23.8)	10.9 (2.7–19.1)	7.3 (0.4–14.2)	68.3 (56.5–80.1)	15.0 (6.0–24.0)	11.7 (3.6–19.8)	5.0 (0.0–10.5)
2-Methoxycinnamaldehyde	0.009 (0.09)	0.823	87.3 (80.6–94.0)	6.4 (0.4–12.4)	2.7 (0.0–6.4)	3.6 (0.0–8.5)	89.5 (82.0–97.0)	4.8 (0.0–10.1)	3.8 (0.0–7.9)	1.9 (0.0–5.5)
	0.09 (0.90)	0.780	89.8 (82.3–97.3)	5.1 (0.1–10.1)	4.1 (0.0–8.1)	1.0 (0.0–3.5)	86.0 (77.0–95.0)	5.6 (0.2–11.0)	5.6 (0.2–11.0)	2.8 (0.0–6.8)
Cumin[c]	0.1	<0.001	77.0 (67.4–86.6)	16.2 (7.8–24.6)	5.4 (0.2–10.6)	1.4 (0.0–4.0)	0.0 (0.0–0.0)	13.2 (5.1–21.3)	75.0 (64.7–85.3)	11.8 (4.1–19.5)
	1	<0.001	79.5 (70.2–88.8)	11.0 (3.8–18.2)	6.8 (1.0–12.6)	2.7 (0.0–6.4)	0.0 (0.0–0.0)	5.2 (0.2–10.2)	80.5 (71.7–89.3)	14.3 (6.5–22.1)
Cuminaldehyde	0.003 (0.03)	0.095	85.7 (76.9–94.5)	7.1 (1.5–12.6)	5.7 (0.3–11.1)	1.4 (0.0–3.8)	70.6 (58.7–82.5)	15.7 (6.6–24.5)	13.7 (6.4–21.0)	0.0 (0.0–0.0)
	0.03 (0.29)	0.009	70.0 (58.4–81.6)	23.3 (12.6–34.0)	5.0 (0.0–10.5)	1.7 (0.0–4.9)	80.0 (70.6–89.4)	10.0 (3.0–17.0)	0.0 (0.0–0.0)	10.0 (3.0–17.0)
	0.3 (2.93)	<0.001	82.0 (72.4–91.6)	11.5 (3.5–19.5)	0.0 (0.0–0.0)	6.6 (0.4–12.8)	46.1 (35.7–56.5)	22.5 (13.8–31.2)	28.1 (18.8–37.4)	3.4 (0.0–7.1)
β-Pinene	0.01 (0.09)	0.986	80.0 (70.9–89.1)	8.0 (1.9–14.1)	6.7 (1.1–12.3)	5.3 (0.2–10.4)	85.2 (76.3–94.1)	8.2 (1.3–15.1)	1.6 (0.0–4.8)	4.9 (0.0–10.3)
	0.10 (0.87)	0.679	76.2 (67.1–85.3)	13.1 (5.9–20.3)	8.3 (2.4–14.2)	2.4 (0.0–5.7)	74.7 (65.3–84.1)	13.3 (6.0–20.6)	9.6 (3.3–15.9)	2.4 (0.0–5.7)
γ-Terpinene	0.01 (0.09)	0.429	78.5 (69.4–87.6)	10.1 (3.4–16.8)	8.9 (2.6–15.2)	2.5 (0.0–6.0)	90.2 (82.7–97.7)	1.6 (0.0–4.8)	8.2 (1.3–15.1)	0.0 (0.0–0.0)
	0.10 (0.85)	0.105	90.5 (83.8–97.2)	5.4 (0.2–10.6)	4.1 (0.0–8.6)	0.0 (0.0–0.0)	82.6 (73.7–91.5)	8.7 (2.1–15.3)	5.8 (0.3–11.3)	2.9 (0.0–6.9)
p-Cymene	0.01 (0.09)	0.126	75.8 (67.0–84.6)	9.9 (3.8–16.0)	12.1 (5.4–18.8)	2.2 (0.0–5.2)	82.9 (74.4–91.4)	5.3 (0.3–10.3)	10.5 (3.6–17.4)	1.3 (0.0–3.9)
	0.10 (0.86)	0.679	69.2 (58.0–80.4)	18.5 (9.1–27.9)	12.3 (4.3–20.3)	0.0 (0.0–0.0)	56.0 (44.8–67.2)	18.7 (9.9–27.5)	25.3 (15.5–35.1)	0.0 (0.0–0.0)
Citronella[d]	0.1	<0.001	73.4 (63.7–83.1)	17.7 (9.3–26.1)	7.6 (1.8–13.4)	1.3 (0.0–3.8)	0.0 (0.0–0.0)	7.1 (0.4–13.8)	73.2 (61.6–84.8)	19.6 (9.2–30.0)
	1	<0.001	80.3 (71.0–89.6)	9.9 (3.0–16.8)	9.9 (3.0–16.8)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	2.9 (0.0–6.8)	48.6 (36.9–60.3)	48.6 (36.9–60.3)
Citronellal	0.034 (0.29)	0.852	78.0 (66.5–89.5)	10.0 (1.7–18.3)	6.0 (−0.6–12.6)	6.0 (0.0–12.6)	78.0 (66.5–89.5)	6.0 (0.0–12.6)	6.0 (−0.6–12.6)	10.0 (1.7–18.3)
	0.34 (2.91)	<0.001	71.7 (59.6–83.8)	9.4 (1.5–17.3)	11.3 (2.8–19.8)	7.5 (0.4–14.6)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	17.5 (7.6–27.4)	82.5 (72.6–92.4)
Geraniol	0.002 (0.02)	0.269	79.0 (69.8–88.2)	13.6 (6.4–20.8)	7.4 (0.3–14.5)	0.0 (0.0–0.0)	80.8 (71.3–90.3)	9.1 (1.4–16.8)	6.1 (0.0–12.6)	4.0 (0.0–8.1)
	0.023 (0.20)	0.005	54.3 (43.5–65.1)	12.3 (5.1–19.5)	30.9 (20.8–41.0)	2.5 (0.0–5.9)	55.9 (44.1–67.7)	10.3 (3.1–17.5)	16.2 (7.4–25.0)	17.6 (8.5–26.7)
	0.23 (2.05)	<0.001	63.9 (52.8–75.0)	9.7 (2.9–16.5)	18.1 (9.2–27.0)	8.3 (1.9–14.7)	0.0 (0.0–0.0)	3.0 (0.0–7.1)	86.4 (78.1–94.7)	10.6 (3.2–18)
Citronellol	0.012 (0.10)	0.427	73.6 (63.4–83.8)	12.5 (4.9–20.1)	8.3 (1.9–14.7)	5.6 (0.3–10.9)	81.5 (72.1–90.9)	4.6 (0.0–9.7)	7.7 (1.2–14.2)	6.2 (0.4–12.0)
	0.12 (1.03)	<0.001	69.8 (58.5–81.1)	17.5 (8.1–26.9)	7.9 (1.2–14.6)	4.8 (0.0–10.1)	0.0 (0.0–0.0)	11.3 (2.8–19.8)	62.3 (49.2–75.4)	26.4 (14.5–38.3)
Geranyl acetate	0.04 (0.37)	0.182	83.9 (74.7–93.1)	6.5 (0.4–12.6)	6.5 (0.4–12.6)	3.2 (0.0–7.6)	83.0 (72.9–93.1)	15.1 (5.5–24.7)	1.9 (0.0–5.6)	0.0 (0.0–0.0)
	0.40 (3.66)	<0.001	86.0 (75.6–96.4)	9.3 (0.6–18.0)	4.7 (0.0–11.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	6.7 (0.4–13.0)	68.3 (56.5–80.1)	25.0 (14.0–36.0)

Table 4. Continued

Product	Concentration (%) (mg L^{-1})	*P*-value[a]	Control				Treated			
			Top (%) (CI)	Middle (%) (CI)	Bottom (%) (CI)	Dead (%) (CI)	Top (%) (CI)	Middle (%) (CI)	Bottom (%) (CI)	Dead (%) (CI)
Limonene	0.003 (0.03)	0.793	84.4 (76.0–92.8)	9.1 (1.4–16.8)	3.9 (0.0–8.0)	2.6 (0.0–6.2)	79.8 (70.4–89.2)	8.9 (2.6–15.2)	5.1 (0.0–10.9)	6.3 (0.4–12.2)
	0.03 (0.25)	0.011	70.9 (58.9–82.9)	7.3 (0.4–14.2)	7.3 (0.4–14.2)	14.5 (5.2–23.8)	74.1 (64.8–83.4)	9.4 (3.2–15.6)	15.3 (7.6–23)	1.2 (0.0–3.5)
	0.3 (2.52)	0.045	91.1 (83.6–98.6)	5.4 (0.0–11.3)	0.0 (0.0–0.0)	3.6 (0.0–8.5)	82.9 (74.1–91.7)	10.0 (3.0–17.0)	7.1 (1.1–13.1)	0.0 (0.0–0.0)
Lemongrass[e]	0.1	<0.001	78.7 (69.4–88.0)	17.3 (8.7–25.9)	1.3 (0.0–3.9)	2.7 (0.0–6.3)	0.0 (0.0–0.0)	7.1 (1.1–13.1)	84.3 (75.8–92.8)	8.6 (2–15.2)
	1	<0.001	81.3 (72.5–90.1)	10.7 (3.7–17.7)	6.7 (1.1–12.3)	1.3 (0.0–3.9)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	6.5 (1.0–12.0)	93.5 (88–99)
Citral	0.08 (0.71)	0.772	70.3 (59.1–81.5)	7.8 (1.2–14.4)	17.2 (8.0–26.4)	4.7 (0.0–9.9)	77.0 (67.4–86.6)	5.4 (0.2–10.6)	12.2 (4.8–19.6)	5.4 (0.2–10.6)
	0.8 (7.14)	<0.001	86.0 (77.0–95.0)	1.8 (0.0–5.2)	7.0 (0.4–13.6)	5.3 (0.0–11.1)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	5.4 (0.0–11.3)	94.6 (88.7–100.5)

[a] *P*-values determined with Fisher's exact test.
[b] Cinnamon mixture: 78.51% cinnamaldehyde, 9.65% 2-methoxycinnamaldehyde, 3.15% cinnamyl acetate.
[c] Cumin mixture: 30.09% cuminaldehyde, 12.19% *β*-pinene, 11.59% *γ*-terpinene, 9.74% *p*-cymene.
[d] Citronella mixture: 34.74% citronellal, 22.50% geraniol, 12.03% citronellol, 3.51% geranyl acetate, 3.34% limonene.
[e] Lemongrass mixture: 74.08% citral, 4.5% geraniol.

Table 5. Synthesis of behavioural bioassays

Product	Property[e]			
	Repellent	Irritant	Toxic (4 h)	Toxic (24 h)
Citronellal	0	0	+	+
Geraniol	+++	NT	+	++
Citronellol	+	NT	+	++
Geranyl acetate	+	0	0	0
Limonene	++	NT	+	0
Citronella blend[a]	+	NT	+	0
Cuminaldehyde	+	0	+	++
β-Pinene	0	NT	0	0
γ-Terpinene	0	NT	+	0
p-Cymene	0	NT	0	+
Cumin blend[b]	++	NT	+	+
(*E*)-Cinnamaldehyde	+++	0	+	0
2-Methoxycinnamaldehyde	0	0	0	0
Cinnamyl acetate	0	0	++	+
Cinnamon blend[c]	+	NT	+	0
Citral	0	0	+	0
Geraniol	+++	NT	+	++
Geranyl acetate	+	NT	0	0
Lemongrass blend[d]	+	NT	+	+

[a] Citronella mixture: 34.74% citronellal, 22.50% geraniol, 12.03% citronellol, 3.51% geranyl acetate, 3.34% limonene.
[b] Cumin mixture: 30.09% cuminaldehyde, 12.19% *β*-pinene, 11.59% *γ*-terpinene, 9.74 *p*-cymene.
[c] Cinnamon mixture: 78.51% (*E*)-cinnamaldehyde, 9.65% 2-methoxycinnamaldehyde, 3.15% cinnamyl acetate.
[d] Lemongrass mixture: 74.08% citral, 4.5% geraniol, 3.9% geranyl acetate.
[e] + = one *P*-value determined with Fisher's exact test was significant; 0 = no *P*-values determined with Fisher's exact-test were significant; NT = not tested.

repellents could actually function by activating olfactory receptor neurons,[44] while irritants could activate gustatory receptor neurons on tarsi.[45] Two pathways regarding their toxic properties have been studied: the inhibition of cholinesterase and interference with the neuromodulator octopamine and with GABA-gated chloride channels.[28,46,47] It is thus essential to gain further insight into the bioactive compound(s) and their biological actions so as to be able to consider their overall repellent and/or insecticidal potential.

Essential oils are complex blends of several molecules. This is one of the first studies to link the behavioural effects of essential oils, mixtures of major compounds and single major compounds. In our previous study, essential oils of *Cymbopogon citratus*, *Cymbopogon winterianus*, *Cuminum cyminum* and *Cinnamomum zeylanicum* were repellent at 1–10%, and they also showed toxic effects at 1%, i.e. 96.3% mortality for cinnamon oil, 64.7% for citronella oil, 61.0% for lemongrass oil and 30.0% for cumin oil (Deletre E and Mallent M *et al.* accepted for publication).[31] In the repellency assay, the four mixtures of major compounds were repellent, like their associated essential oils. Among the cinnamon mixture compounds, only cinnamaldehyde was repellent, so the repellent effect of cinnamon essential oils would certainly be due to cinnamaldehyde. In the same way, cuminaldehyde could be responsible for the repellent effect of the cumin mixture. Among the citronella mixture compounds, geraniol, citronellol and

geranyl acetate were repellent. This finding suggests that the repellent effect of the citronella mixture was due to an additive effect of all of these compounds. Moreover, citral, geraniol and geranyl acetate could be responsible for the repellent effect of the lemongrass mixture. The repellent effect of essential oils was due to one major compound or several major compounds. In the toxicity assay, cinnamaldehyde alone was as toxic as the cinnamon mixture, contrary to the other major compounds, so the cinnamon toxicity was certainly due to cinnamaldehyde. Cuminaldehyde and γ-terpinene were also toxic, but they were less toxic than the cumin mixture, so a synergetic effect between these two compounds or other minor compounds could explain the toxic effect. Citronellol, citronellal and geraniol were also toxic, but not as efficient as the citronella mixture. In addition, citral and geraniol were toxic but not as toxic as the lemongrass mixture. A synergistic effect between compounds could explain these results.

Except for cinnamaldehyde, the whitefly net-crossing rate of the single compounds was higher than that of their associated mixtures, i.e. a single compound was less efficient as an olfactory barrier than its associated mixture. Moreover, after 4 h, the toxicity of these single compounds was lower than that of their associated mixtures, except for cinnamaldehyde. This suggested that the major compounds of cumin, citronella and lemongrass essential oils had synergistic/additive effects. Conversely, the repellency of the mixtures appeared to be due to one or several major compounds. For example, the cinnamon mixture was repellent, and only cinnamaldehyde was repellent alone and as repellent as the mixture. We obtained the same result with the cumin mixture and cuminaldehyde, but this was not always the case, as all the major compounds of citronella and lemongrass were repellent at higher concentration. In many studies, the activity of an essential oil against insects is explained by the major compounds.[29] However, the activity of the main compounds could be modulated by other minor molecules.[48–50] Many of the essential oil compounds are actually involved in cell penetration, lipophilic or hydrophilic attraction and fixation on cell walls and membranes, with the cellular distribution determining the different types of radical reaction produced.[51] Our results showed that the biological effect of an essential oil is not always due to the activity of the major compound alone. Indeed, synergistic effects may occur between the major or minor compounds.

This study also aimed to determine the most promising compounds of four essential oils (cumin, cinnamon, citronella and lemongrass) for pest control applications. The most promising compounds for net treatments were cinnamaldehyde, limonene, citronellol, citronellal, citral and geraniol because their associated whitefly net-crossing rates were low. Repellency includes every phenomenon that prevents a pest from tracking, locating and/or recognising its host (Deletre E, unpublished). Repellents at a distance, i.e. olfactory-mediated effect, and contact repellents or irritants, i.e. contact-mediated effect, are two different phenomena that could be usefully combined with insect-proof netting. Three compounds, i.e. cinnamaldehyde, citronellol and geraniol, were repellent at a distance according to the repellence bioassays, but no compounds showed any irritant effects. Among these compounds, cinnamaldehyde showed the highest toxicity (100%), and we know that whiteflies develop rapid resistance, so with the aim of having a sustainable strategy this product is not the most promising. Geraniol and citronellol could thus be the most promising compounds in combination with netting, although these compounds caused 32.1 and 17.1% mortality after 24 h.

The laboratory findings indicated a procedural direction and screening methods for products that have potential for whitefly control owing to their repellent and toxic effects. These products (geraniol and citronellol) could be used in insect-proof net treatment, and this strategy has already shown good results in the field with chemical products. For example, Martin *et al.*[19,52] showed that a net treated with alpha-cypermethrin blocked the pest *Myzus persicae* on cabbage crops in field conditions because of the irritant and repellent effects of this chemical. Moreover, a net treated with alpha-cypermethrin was shown to reduce the whitefly net-crossing rate and improve tomato crop protection as compared with untreated netting.[20] However, there are also approaches other than applying chemicals on nets to repel whiteflies, e.g. intercropping. Indeed, a repellent plant – as a natural diffusor of repellent volatile – can be combined with a net to obtain a repellent effect or to confuse the pest, thereby reducing the amount of chemicals needed for crop protection. Our findings suggested that plants that produce relatively high amounts of geraniol and/or citronellol volatiles could be of interest in investigating intercropping strategy. However, the plants have to match the requirements for intercropping, for example the cultural conditions of the crop. Mansour *et al.*[53] have already shown that intercropping tomato plants with okra, *A. esculentus*, and brinjal, *S. melongena*, decreased the whitefly infestation rate as compared with monocropped tomato. One potential limit of this concept is that the exact mode of repellency and irritancy action was not studied. Togni *et al.*[54] also showed that coriander (*Coriandrum sativum*) volatiles masked tomato volatiles in host plant selection. Tosh and Brogan[55] also put forward the idea of supplying whiteflies with a superabundance of volatiles to confuse this generalist insect. In conclusion, this study identified volatile candidates that may be emitted by companion plants or by diffusers, e.g. chemically impregnated nets, to repel whiteflies. These compounds could be used alone or in mixtures to establish an olfactory barrier as a supplement to the visual and physical barrier of an insect-proof net in order to protect vegetables.

ACKNOWLEDGEMENTS

We thank CIRAD, IRD and Mutavie, Paris, France, for supporting this work. The funding sources had no involvement at any stage in the preparation of this paper. Our thanks to Cica Urbino from CIRAD, who identified the biotype Q2 of the *Bemisia tabaci* used in this study.

REFERENCES

1 Jing Y, Huang J, Ma RY and Han JC, Host plant preferences of *Bemisia tabaci* Gennadius. *Entomol Sin* **10**(2):109–114 (2003).
2 Berlinger MJ, Host plant resistance to *Bemisia tabaci*. *Agric Ecosyst Environ* **17**:69–82 (1986).
3 Blackmer JL and Byrne DN, Flight behaviour of *Bemisia tabaci* in a vertical flight chamber: effect of time of day, sex, age and host quality. *Physiol Entomol* **18**(3):223–232 (1993).
4 Cohen S and Berlinger MJ, Transmission and cultural control of whitefly-borne viruses. *Agric Ecosyst Environ* **17**:89–97 (1986).
5 Jones DR, Plant viruses transmitted by whiteflies. *Eur J Plant Pathol* **10**(3):195–219 (2003).
6 Muniz M, Nombela G and Barrios L, Within-plant distribution and infestation pattern of the B- and Q-biotypes of the whitefly, *Bemisia tabaci*, on tomato and pepper. *Entomol Exp Applic* **104**:369–373 (2002).
7 Zhang W, McAuslane HJ and Schuster DJ, Repellency of ginger oil to *Bemisia argentifolii* (Homoptera: Aleyrodidae) on tomato. *J Econ Entomol* **97**(4):1310–1318 (2004).

8 Denholm I, Cahill M, Dennehy TJ and Horowitz AR, Challenges with managing insecticide resistance in agricultural pests, exemplified by the whitefly *Bemisia tabaci*. *Phil Trans R Soc Lond Biol Sci* **353**:1757–1767 (1998).
9 Elbert A and Nauen R, Resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides in southern Spain with special reference to neonicotinoids. *Pest Manag Sci* **56**(1):60–64 (2000).
10 Palumbo JC, Horowitz AR and Prabhaker N, Insecticidal control and resistance management for *Bemisia tabaci*. *Crop Prot* **20**(9):739–765 (2001).
11 Roditakis E, Grispou M, Morou E, Kristoffersen JB, Roditakis N, Nauen R *et al.*, Current status of insecticide resistance in Q biotype *Bemisia tabaci* populations from Crete. *Pest Manag Sci* **65**(3):313–322 (2009).
12 Houndété TA, Kétoh GK, Hema OSA, Brévault T, Glitho IA and Martin T, Insecticide resistance in field populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in West Africa. *Pest Manag Sci* **66**:1181–1185 (2010).
13 Houndété TA, Fournier D, Ketoh GK, Glitho IA, Nauen R and Martin T, Biochemical determination of acetylcholinesterase genotypes conferring resistance to the organophosphate insecticide chlorpyriphos in field populations of *Bemisia tabaci* from Benin, West Africa. *Pest Biochem Physiol* **98**:115–120 (2010).
14 Gnankiné O, Mouton L, Savadogo A, Martin T, Sanon A, Dabire RK *et al.*, Biotype status and resistance to neonicotinoids and carbosulfan in *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Burkina Faso, West Africa. *Int J Pest Manag* **59**(2):95–102 (2013).
15 Wang Z, Yan H, Yang Y and Wu Y, Biotype and insecticide resistance status of the whitefly *Bemisia tabaci* from China. *Pest Manag Sci* **66**(12):1360–1366 (2010).
16 Perring TM, The *Bemisia tabaci* species complex. *Crop Prot* **20**:725–737 (2001).
17 Byrne FJ and Devonshire AL, Biochemical evidence of haplodiploidy in the whitefly *Bemisia tabaci*. *Biochem Genet* **34**:93–107 (1996).
18 Weintraub PG, Physical control: an important tool in pest management programs, in *Biorational Control of Arthropods Pests*, ed. by Ishaaya I and Horowitz AR. Springer Science, Amsterdam, The Netherlands, pp. 317–324 (2009).
19 Martin T, Palix R, Kamal A, Deletre E, Bonafos R, Simon S *et al.*, A repellent treated netting as a new technology for protecting vegetable crops. *J Econ Entomol* **106**(4):1699–1706 (2013).
20 Martin T, Gogo EO, Saidi M, Kamal A, Deletre E, Bonafos R *et al.*, Repellent effect of an alphacypermethrin treated net against the whitefly *Bemisia tabaci* Gennadius. *J Econ Entomol* **107**(2):684–690 (2014).
21 Regnault-Roger C, Philogene BJR and Vincent C, *Biopesticide d'Origine Végétale*. Tec & Doc Lavoisier, Paris, 546 pp. (2002).
22 Hilje L and Mora GA Promissory botanical repellents/deterrents for managing two key tropical insect pests, the whitefly *Bemisia tabaci* and the mahogany shootborer *Hypsipyla grandella*, in *Naturally Occurring Bioactive Compounds*, ed. by Rai M and Carpinella MC. Elsevier Science, London, UK, pp. 379–403 (2006).
23 Amer A and Mehlorn H, Repellency effect of forty one essential oils against *Aedes*, *Anopheles*, and *Culex* mosquitoes. *Parasitol Res* **99**:478–490 (2006).
24 Boer H, Vongsombath C, Palsson K, Bjork L and Jaenson TGT, Botanical repellents and pesticides traditionally used against hematophagous inverterbrates in Lao People's Democratic Republic: a comparative study of plant used in 66 villages. *J Med Entomol* **47**(3):400–414 (2010).
25 Nerio LS, Olivero-Verbel J and Stashenko E, Repellent activity of essential oils: a review. *Bioresour Technol* **101**:372–378 (2010).
26 Maia FM and Moore SJ, Plant-based insect repellents: a review of their efficacy, development and testing. *Malaria J* **10**:S11 (2011).
27 Zoubiri S and Baalioumer A, Potentiality of plants as source of insecticide principles. *J Saudi Chem Soc* **18**(6):925–938 (2014).
28 Regnault-Roger C, Vincent C and Arnasson JT, Essential oil in insect control: low-risk products in high-stakes world. *Annu Rev Entomol* **57**:405–424 (2012).
29 Ipek E, Zeytinoglu H, Okay S, Tuylu BA, Kurkcuoglu M and Husnu Can Baser K, Genotoxicity and antigenotoxicity of origanum oil and carvacrol evaluated by *Ames Salmonella*/microsomal test. *Food Chem* **93**:551–556 (2005).
30 Moore SJ, Lenglet A and Hill N, Plant-based insect repellents, in *Insect Repellents: Principles, Methods and Uses*, ed. by Debboun M, Frances SP and Strickman D. CRC Press, Boca Raton, FL, Ch.14, pp. 275–303 (2007).
31 Deletre E, Mallent M, Menut C, Chandre F and Martin T, Behavioral response of *Bemisia tabaci* to 20 plant extracts. *J Econ Entomol* (accepted).
32 Deletre E, Martin T, Campagne P, Bourguet D, Cadin A, Menut C *et al.*, Repellent, irritant and toxic effects of 20 plant extracts on adults of the malaria vector *Anopheles gambiae* mosquito. *PLoS ONE* **8**(12):e82103 (2013).
33 Adams RP, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th Edition. Allured Publishing Corporation, Carol Stream, IL, 804 pp. (2007).
34 Noldus LPJ, Spink JAJ and Tegelenbosch RAJ, Computerised video tracking, movement analysis and behaviour recognition in insects. *Comput Electron Agric* **35**:201–227 (2002).
35 *R: A Language and Environment for Statistical Computing*. [Online]. R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria. Available:http://www.R-project.org/ [05 March 2015].
36 Holm S, A simple sequential rejective multiple test procedure. *Scand J Stat* **6**:65–70 (1979).
37 Püntener W, *Manual for Field Trials*, 2nd edition. Agricultural Division, Ciba-Geigy Limited, Basle, Switzerland (1981).
38 Müller GC, Junnila A, Butler J, Kravchenko VD, Revay EE, Weiss RW *et al.*, Efficacy of the botanical repellents geraniol, linalool, and citronella against mosquitoes. *J Vect Ecol* **34**(1):2–8 (2009).
39 Revay EE, Junnila A, Kline DL, Xue RD, Bernier UR, Kravchenko VD *et al.*, Reduction of mosquito biting pressure by timed-release 0.3% aerosolized geraniol. *Acta Tropica* **124**(1):102–105 (2012).
40 Baldin ELL, Aguiar GP, Fanela TLM, Soares MCE, Groppo M and Crotti AEM, Bioactivity of *Pelargonium graveolens* essential oil and related monoterpenoids against sweet potato whitefly, *Bemisia tabaci* biotype B. *J Pest Sci* **86**(2):301–308 (2014).
41 Tunón H, Thorsell W, Mikiver A and Malander I, Arthropod repellency, especially tick (*Ixodes ricinus*), exerted by extract from *Artemisia abrotanum* and essential oil from flowers of *Dianthus caryophyllum*. *Fitoterapia* **77**:257–261 (2006).
42 Regnault-Roger C, The potential of botanical oils for insect pest control. *Integr Pest Manag Sci* **2**:25–34 (1997).
43 Huang Y and Ho SH, Toxicity and antifeedant activities of cinnamaldehyde against the grain storage insects, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. *J Stored Prod Res* **34**:11–17 (1998).
44 Dekker T, Ignell R, Ghebru M, Glinwood R and Hopkins R, Identification of mosquito repellent odours from *Ocimum forskolei*. *Parasit Vect* **4**:183–190 (2011).
45 Vosshall LB and Stocker RF, Molecular architecture of smell and taste in Drosophila. *Annu Rev Neurosci* **30**:505–533 (2007).
46 Isman MB, Plant EO for pest and disease management. *Crop Prot* **19**(8–10):603–608 (2000).
47 Isman MB, Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu Rev Entomol* **51**:45–66 (2006).
48 Franzios G, Mirotsou M, Hatziapostolou E, Kral J, Scouras ZG and Mavragani-Tsipidou P, Insecticidal and genotoxic activities of mint essential oils. *J Agric Food Chem* **45**:2690–2694 (1997).
49 Santana-Rios G, Orner GA, Amantana A, Provost C, Wu SY and Dashwood RH, Potent antimutagenic activity of white tea in comparison with green tea in the *Salmonella* assay. *Mutat Res* **495**:61–74 (2001).
50 Hoet S, Stévigny C, Hérent MF and Quetin-Leclercq J, Antitrypanosomal compounds from leaf essential oil of *Strychnos spinosa*. *Planta Med* **72**:480–482 (2006).
51 Bakkali F, Averbeck S, Averbeck D and Idaomar M, Biological effects of essential oils – a review. *Food Chem Toxicol* **46**:446–475 (2008).
52 Martin T, Assogba-Komlan F, Houndete T, Hougard J M and Chandre F, Efficacy of mosquito netting for sustainable small holders' cabbage production in Africa. *J Econ Entomol* **99**(2):450–454 (2006).
53 Mansour SAA, Roff MN, Saad KA, Abuzid I and Idris AB, Responses of whitefly *Bemisia tabaci* (Genn) (Homoptera: Aleyrodidae) population on tomato *Lycopersicon esculentum* mixed with other crops under glasshouse conditions. *APCBEE Proc* **4**:48–52 (2012).
54 Togni PH, Laumann RA, Medeiros MA and Sujii ER, Odour masking of tomato volatiles by coriander volatiles in host plant selection of *Bemisia tabaci* biotype B. *Entomol Exp Applic* **136**(2):164–173 (2010).
55 Tosh CR and Brogan B, Control of tomato whiteflies using the confusion effect of plant odours. *Agron Sustain Dev* **35**:183–193 (2015).

