ORIGINAL CONTRIBUTION

JOURNAL OF APPLIED ENTOMOLOGY WILEY

Repellent activity of *Cymbopogon citratus* and *Tagetes minuta* and their specific volatiles against *Megalurothrips sjostedti*

Seydou Diabate[1,2,3] | Thibaud Martin[1,2,4] | Lucy Kananu Murungi[3] |
Komi K. M. Fiaboe[1,5] | Sevgan Subramanian[1] | John Wesonga[3] | Emilie Deletre[1,2,4]

[1]International Centre of Insect Physiology and Ecology, Nairobi, Kenya

[2]CIRAD, UPR Hortsys, Université de Montpellier, Montpellier, France

[3]Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

[4]Université Montpellier, Montpellier, France

[5]IITA-Cameroon, Yaoundé, Cameroon

Correspondence
Seydou Diabate, International Centre of Insect Physiology and Ecology, P.O. Box 30772-00100, Nairobi, Kenya.
Emails: seydoudiabate01@gmail.com; sdiabate@icipe.org

Abstract

Cowpea is an important source of protein for people in Africa. However, the crop suffers major damage and yield losses due to bean flower thrips, *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae). Although companion plants are known to reduce the damage caused by insect pests, the role of their volatiles in repelling pests from target plants has been the subject of few investigations. Here, we used the Y-tube olfactometer experiments and chemical analyses to investigate the effect of volatiles from cowpea flowers and two companion plants; lemongrass, *Cymbopogon citratus* and Mexican marigold, *Tagetes minuta* on the olfactory responses of *M. sjostedti*. The results revealed that *M. sjostedti* males and females were repelled by the volatiles from freshly cut leaves of *C. citratus*. The combination of freshly cut leaves of *C. citratus* and cowpea flower was repellent to females but not to males. The female thrips, but not males, were repelled by the volatiles from the vegetative stage of *T. minuta*. Fifty-four compounds were identified in the volatiles from two herbal plants. Among the major compounds, citral and a 4-component blend comprised of dihydrotagetone, (*Z*)-3-hexenyl acetate, limonene and (*Z*)-*β*-ocimene repelled females but dihydrotagetone alone attracted females. While myrcene combined with cowpea flower volatiles enhanced the attraction of females *M. sjostedti*, when tested alone was not attractive. These results highlight the potential of volatiles from *C. citratus* and *T. minuta* to repel *M. sjostedti* females. The use of these plants as companion plants in a cowpea cropping system could reduce *M. sjostedti* infestation.

KEYWORDS
companion plant, cowpea, lemongrass, marigold, thrips

1 | INTRODUCTION

Cowpea, *Vigna unguiculata* L., is an important source of proteins for human nutrition and livestock feed in Africa (Singh, Ajeigbe, Tarawali, Fernandez-Rivera, & Abubakar, 2003; Togola et al., 2017). Annual production of cowpea grains was estimated at 6.7 million metric

tons in 2016 (FAOSTAT, 2016). Around 200 million people consume cowpea every day in Africa (Popelka, Gollasch, Moore, Molvig, & Higgins, 2006). However, cowpea crops are threatened by several insect pests, including *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae) (Abtew, 2015; OECD, 2015). The bean flower thrips, *M. sjostedti*, is a major pest of leguminous plants, especially cowpea in Africa and it can cause between 20% and 100% cowpea pod yield losses without the use of synthetic pesticides (Abtew, 2015;

The work was conducted at International Centre of Insect Physiology and Ecology (*icipe*)

Ekesi, Maniania, & Onu, 1999). Their small size, cryptic feeding habits, ability to pupate in the soil and fast development make them difficult to be controlled with pesticides (Abtew, 2015). Additionally, chemical pesticides are costly for small-scale farmers, have a negative effect on beneficial insects and are harmful to the environment and human health (Abtew et al., 2016; de Bon et al., 2014). As such, alternative environmentally friendly methods to reduce pest populations on crops are required. The use of companion plants producing repellent compounds is one possible pesticide-free alternative for the control of *M. sjostedti*.

The potential of repellent companion plants to control insect pests has been demonstrated by several authors (Parker, Snyder, Hamilton, & Rodriguez-Saona, 2013; Parolin et al., 2012). For example, *Ocimum basilicum* L. (basil) has been used to reduce populations of thrips (Parker et al., 2013), aphids (Basedow, Hua, & Aggarwal, 2006) and pink bollworm (Schader, Zaller, & Köpke, 2005). However, numerous failures have been reported on the efficacy of non-host volatiles to reduce insect pests in the field (Moreau, Warman, & Hoyle, 2006; Webster & Cardé, 2016). For example, the intercropping of the non-host plant French marigold with host potato plants did not reduce the population of the Colorado potato beetle, *Leptinotarsa decemlineata* Say, but rather increased the pest attack (Moreau et al., 2006). The identification of one or a blend of repellent plant volatiles could help us to better select companion plants to be used as an olfactory barrier to prevent *M. sjostedti* infestation in cowpea.

The Cymbopogon and Tagetes genera have been shown to have a broad spectrum of activity against many arthropods (Nerio, Olivero-Verbel, & Stashenko, 2010; Singh et al., 2015). Lemongrass, *Cymbopogon citratus* (DC.) Stapf, extracts have been shown in previous studies to efficiently repel *M. sjostedti* female thrips (Abtew, 2015). However, the short repellence duration of extracts of *C. citratus* remains a major problem in the control of insect pests (Nerio et al., 2010). Whole lemongrass emits small amounts of volatiles compared to when it is cut or crushed. The repellence duration of volatiles from *C. citratus* on *M. sjostedti* was evaluated using freshly cut and old cut leaves of *C. citratus*. Tagetes oil is described by the United States Environmental Agency as a biochemical pesticide that can be used for the control of thrips (USEPA, 2012). However, little is known about the role of volatiles from *C. citratus* and Mexican marigold *Tagetes minuta* L in controlling *M. sjostedti*. In addition, the repellent behaviour and its controlling mechanisms are complex (Deletre et al., 2016). Understanding the mechanisms involved in the repellent effect of volatiles from companion plants would improve our knowledge of pest control and the use of such plants. Thus, the aim of the present study was to evaluate the repellent effect of *C. citratus* and *T. minuta* plants and their major compounds against male and female *M. sjostedti*. We hypothesized that volatiles from *T. minuta* and *C. citratus* plants repel *M. sjostedti*. We tested our hypothesis using behavioural assays and chemical analysis (a) to evaluate the response of *M. sjostedti* to the two plants alone or in combination with cowpea plants, (b) to identify the volatile compounds from the two plants that mediate the behavioural response of thrips and (c)

to evaluate the effect of individual compounds or a blend of major volatile compounds from both plants on *M. sjostedti* behaviour.

2 | MATERIALS AND METHODS

2.1 | Cultivation of test plants

Cowpea, *V. unguiculata* var. Ken Kunde 1 (seeds purchased from Simlaw Seeds Company Limited), Mexican marigold, *Tagetes minuta*, (seeds collected at the Kenya Agricultural and Livestock Research Organization (KALRO) Industrial Crops Research Centre (ICRC), Mwea (0°37′09.0′′S 37°22′09.4′′E) and lemongrass, *C. citratus*, (seedlings purchased from Simlaw Seeds Company Limited) were established in a screen house (27 ± 7°C temperature, 75 ± 5% relative humidity; 12L:12 D photoperiod) at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya (1°13′17.9′′S 36°53′48.1′′E). Individual plants were grown free from pesticides and watered regularly. Cattle manure was added to the soil. Potted eight-week-old cowpea seedlings with open flowers were used in the experiments. In our previous study, we found that the open flowers of cowpea var. Ken Kunde 1 were an attractant to female *M. sjostedti* (Diabate et al., 2019); therefore, this cultivar was used in the current study. Preliminary results showed that *C. citratus* produces more citral when the leaves are cut or damaged by insects. For this reason, the repellent effect of *C. citratus* was evaluated by cutting leaves into 0.5-cm-wide strips in the laboratory (25 ± 1°C temperature; 40%–50% RH). The cut leaves of *C. citratus* (1 g) were used immediately after cutting (0 hr) as *fresh* leaves and 24 hr after cutting as *old* leaves in all the experiments. The cut leaves were kept in a Petri dish at ambient temperature (25 ± 1°C). The vegetative and flowering stages of 2- to 3-month-old *T. minuta* were used for the experiments.

2.2 | Rearing of test insects

Bean flower thrips, *M. sjostedti*, were reared in the Animal Rearing and Containment Unit (ARCU) at *icipe* at a temperature of 26 ± 2°C, 50%–60% relative humidity and a 12 L:12 D photoperiod. The insects were reared on French bean pods, *Phaseolus vulgaris* L. Every 2 days, bean pods containing fresh thrips eggs were transferred to clean jars to obtain adult thrips of the same age (Abtew et al., 2015; Nyasani, Meyhöfer, Subramanian, & Poehling, 2013). The newly emerged males (≈10 days from oviposition) and females (≈12 days from oviposition) were used in the respective experiments. Male and female *M. sjostedti* were identified visually. The difference between males and females were based on their robustness and thickness of the abdomen (Diabate et al., 2019; Sani & Umar, 2017).

2.3 | Olfactory response of *M. sjostedti* to plant volatiles

A Y-tube olfactometer (0.5 cm internal diameter, 4 cm stem length, 5.5 cm arm length and 50° angle) was used to evaluate the behavioural

response of *M. sjostedti* to headspace volatiles of the test plants in the laboratory (25 ± 1°C temperature; 40%–50% RH). The Y-tube was held in an inclining position at an angle of 25° to the horizontal plane (Koschier, Kogel, & Visser, 2000) and was covered with a cardboard box (50 cm × 48 cm × 33 cm) which was open at the top and illuminated from above by a light source (60 lux) (Diabate et al., 2019). Compressed air from a pump (KnF, Laboport, Legallais) was purified by passing through an activated charcoal filter and humidified via a conical glass flask containing distilled water. Air was pushed into two flow metres at 60 ml/min. The air then passed through two polyester (Nalophan) bags (38 cm high × 25 cm wide, cooking bag, Chevalier Diffusion-F33890 Pessac sur Dordogne, Belgium) containing the odour sources then into each arm of the olfactometer. The cut leaves of *C. citratus* or whole plants of *T. minuta* (the pots were covered with aluminium foil) were placed in the polyester (Nalophan) bag, and the bag closed tightly. The compounds tested were enclosed alone or with the plant in the bag as an odour source. The olfactory responses of male and female *M. sjostedti* were tested on (a) freshly cut leaves of *C. citratus* (1 g) versus clean air; (b) old cut leaves (1 g) of *C. citratus* versus clean air; (c) vegetative *T. minuta* versus clean air; (d) flowering *T. minuta* versus clean air; (e) freshly cut leaves of *C. citratus* + open cowpea flower versus open cowpea flower alone; (f) old cut leaves of *C. citratus* + open cowpea flower versus open cowpea flower alone; (g) vegetative *T. minuta* + open cowpea flower versus open cowpea flower alone; and (h) flowering *T. minuta* + open cowpea flower versus open cowpea flower alone. An individual (male or female) thrips was placed at the stem inlet of the Y-tube with a soft hair brush and allowed to choose one of the arms for a maximum of 3 min. A response was recorded if the insect walked into either of the short Y-tube arms within the allocated time. An insect that did not choose either of the arms within 3 min was recorded as non-responsive. The Y-tube olfactometer was cleaned with 70% ethanol after the passage of each thrips to remove possible traces of pheromones or contaminants. The ethanol in the Y-tube was left to evaporate for 60 s at ambient temperature between each insect tested. To avoid any bias, the Y-tube was alternated, and odour sources were connected to the opposite arm after every five male and female thrips tested, while the respective plant materials were replaced after 10 males and females were tested. Sixty male and female thrips were tested per treatment. Every day, at the end of the bioassays, the Y-tube was cleaned with 70% alcohol, then rinsed with distilled water and baked overnight in an oven at 100°C. The Nalophan bags were baked overnight at 100°C.

2.4 | Collection of volatiles

Volatiles were collected from *T. minuta* at either the vegetative or the flowering stage and from 1 g of cut fresh and old leaves of *C. citratus* with Super Q (30 mg, Analytical Research System) adsorbent traps. Traps were pre-cleaned with 2 ml of hexane (Sigma-Aldrich) and 2 ml of dichloromethane (Sigma-Aldrich) to remove contaminants and then dried in a nitrogen stream. Four replicates were performed for each treatment. Individual plant material was enclosed in a Nalophan

bag for 4 hr. The air provided by a pump was purified in activated charcoal, then passed into the bag at a flow rate of 300 ml/min and pulled out through an adsorbent Super Q trap at 200 ml/min. The difference in flow rates prevented unfiltered air from entering the system (Webster et al., 2008). After volatile collection, the Super Q trap was eluted with 150 µl of dichloromethane, and then, 30 ng/µl of 2-hexadecanol was added to the eluate as an internal standard. Volatile collection was also done from empty oven bags under the same conditions to serve as a negative control. Samples were either analysed immediately or stored at −80°C until use.

2.5 | Analysis of volatiles

Volatiles were analysed using an Agilent Technologies 7890A gas chromatograph equipped with an HP-5 MS capillary column (30 m × 0.25 mm ID × 0.25 µm film thickness) (J & W Scientific) coupled to a 5975C mass spectrometer. A 1-µl aliquot of each eluate was analysed in the splitless mode using helium as a carrier gas at a flow rate of 1.2 ml/min. The oven temperature was held at 35°C for 5 min after injection of the sample, then programmed to increase at 10°C/min to 280°C and maintained at this temperature for 5.5 min. Spectra were recorded at 70 eV in the electron impact (EI) ionization mode. Compounds were identified by comparing the mass spectra data with library data: Adams2 terpenoid/natural product library (Adams, 1995) and National Institute of Standards and Technology (NIST, 2008) (MSD ChemStation F.01.00.1903, MS HP). The retention times of some compounds and mass spectra were compared to those of authentic standards ((Z)-3-hexenyl acetate, nerol, neral, geraniol, geranial, (Z)-β-ocimene, (E)-β-ocimene, limonene, dihydrotagetone, α-pinene and linalool). The retention indices of compounds were calculated relative to n-alkane standards (C8-30).

2.6 | Chemicals

The synthetic standards, including myrcene (purity ≥ 95%), limonene (purity 96%), ocimene mixture (purity ≥ 90%), nerol (purity 98%), geraniol (purity 98%) and citral (geranial, neral) (purity 95%), were purchased from Sigma-Aldrich, France. Dihydrotagetone was purchased from Santa Cruz Biotechnology, France. Hexane (purity ≥ 95%), dichloromethane (purity ≥ 99%) and 2-hexadecanol (purity 99%) were purchased from Sigma-Aldrich, UK. Ethanol (purity ≥ 99.8%) was purchased from Sigma-Aldrich, Germany.

2.7 | Olfactory assay with synthetic standards

We used the same Y-tube olfactometer assay (as described above), to evaluate the repellent effect of major compounds identified in the volatiles from *T. minuta* and *C. citratus*. The compounds were tested either alone or in blends at a concentration of 1% (v/v) in dichloromethane. The blend was formulated to simulate the ratio corresponding to the natural ratio occurring in the plants. The synthetic blend of *C. citratus* contained citral, myrcene, geraniol

and nerol in a ratio of 39:21:8:1, respectively. The blend of vegetative *T. minuta* included dihydrotagetone, (Z)-3-hexenyl acetate, limonene and (Z)-β-ocimene in a ratio of 4:3:1:1, respectively. A 50-μl aliquot of each compound or blend was deposited on a filter paper and left for 30 s at 25 ± 1°C to allow the solvent to evaporate. Filter papers were placed in Polyester (Nalophan) bags connected to the olfactometer arms via PTFE tubing. The negative control consisted of 50 μl dichloromethane only. We tested each compound or blend versus control (solvent) and their combinations versus cowpea flower alone. The 10 treatments tested with compounds from cut leaves of *C. citratus* were as follows: (a) myrcene versus control; (b) nerol versus control, (c) citral versus control; (d) geraniol versus control; (e) blend versus control; (f) myrcene + cowpea flower versus cowpea flower; (g) nerol + cowpea flower versus cowpea flower; (h) citral + cowpea flower versus cowpea flower; (i) geraniol + cowpea flower versus cowpea flower; and (j) blend + cowpea flower versus cowpea flower. The 11 treatments tested with compounds from *T. minuta* were as follows: (a) (+)(R)-limonene versus control; (b) (+)(S)-limonene versus control; (c) ocimene versus control; (d) (Z)-3-hexenyl acetate versus control; (e) dihydrotagetone versus control; (f) blend versus control; (g) limonene + cowpea flower versus cowpea flower; (h) ocimene + cowpea flower versus cowpea flower; (i) (Z)-3-hexenyl acetate + cowpea flower versus cowpea flower; (j) dihydrotagetone + cowpea flower versus cowpea flower; and (k) blend + cowpea flower versus cowpea flower. The filter papers were replaced every 60 min. Individual female thrips tested represented a replicate, and each treatment was complete after 60 females were tested.

2.8 | Statistical analysis

Frequency count data from the olfactometer assays were subjected to a chi-square (χ^2) goodness-of-fit test to evaluate the percentage of *M. sjostedti* which made a choice between the two odours tested. The null hypothesis was that thrips had 50:50 distributions across the two arms of the olfactometer. A non-parametric Mann–Whitney–Wilcoxon test was used to analyse differences in the emission of volatiles between vegetative and flowering *T. minuta* plants and fresh and old leaves of *C. citratus*. Principal component analysis (packages "ade4" (Dray & Dufour, 2007)) was used to show the relationship between the fresh and old cut leaves of *C. citratus* and vegetative and flowering *T. minuta* plants based on the emission of volatile compounds using a graphical approach. All data analyses were implemented in R (R-Development-Core-Team, 2018).

3 | RESULTS

3.1 | Behavioural response of *M. sjostedti* to *C. citratus* and *T. minuta*

Male and female *M. sjostedti* were significantly repelled by the volatiles from freshly cut leaves of *C. citratus* relative to clean air (male: χ^2 = 14.51, df = 1, p < 0.001; female: χ^2 = 11.26, df = 1, p < 0.001) (Figure 1 a). However, no repellent effect was observed with old cut leaves (p > 0.05). More female *M. sjostedti* were repelled by cowpea flower combined with freshly cut leaves of *C. citratus* compared to cowpea flower alone (χ^2 = 6.81, df = 1, p < 0.01). For males, no significant difference in effect was observed between cowpea
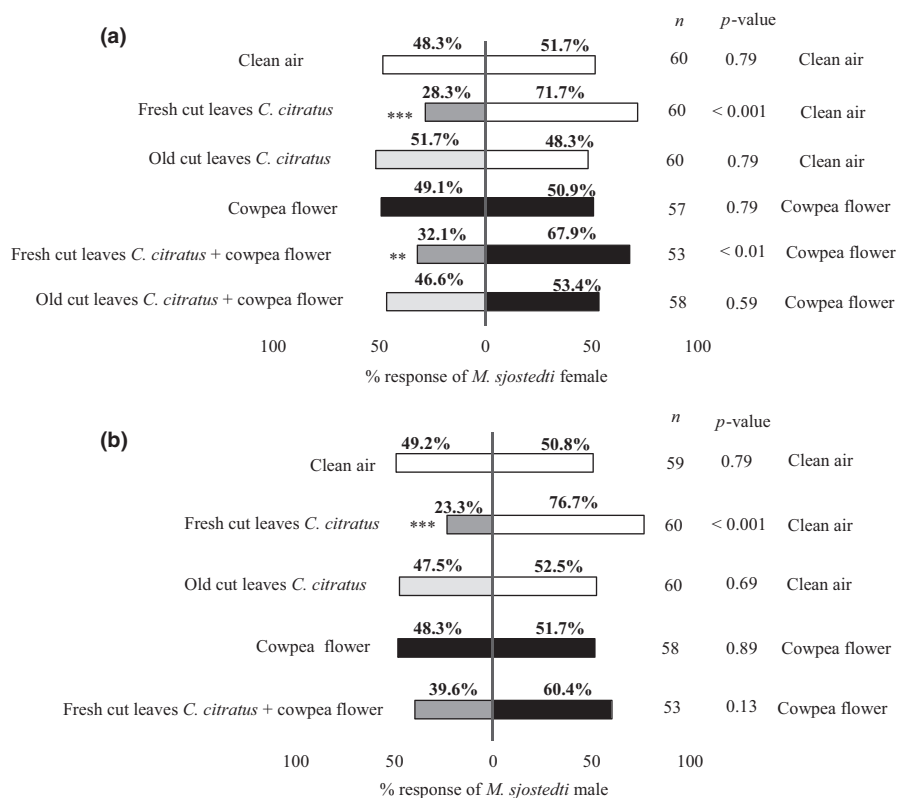

FIGURE 1 Percentage (%) responses of a) female and b) male *M. sjostedti* to freshly and old cut leaves of *C. citratus* versus clean air or in combination versus cowpea flower alone in a Y-tube olfactometer. n = total number of insects which responded per treatment. Significance levels of chi-square tests are indicated by *p < 0.05, **p < 0.01, ***p < 0.001, (χ^2 goodness-of-fit test)

FIGURE 2 Percentage (%) responses of a) female and b) male *M. sjostedti* to vegetative and flowering *T. minuta* versus clean air or in combination versus cowpea flower alone in a Y-tube olfactometer. *n* = total number of insects which responded per treatment. Significance levels of chi-square tests are indicated by *$p < 0.05$, **$p < 0.01$, (χ^2goodness-of-fit test)
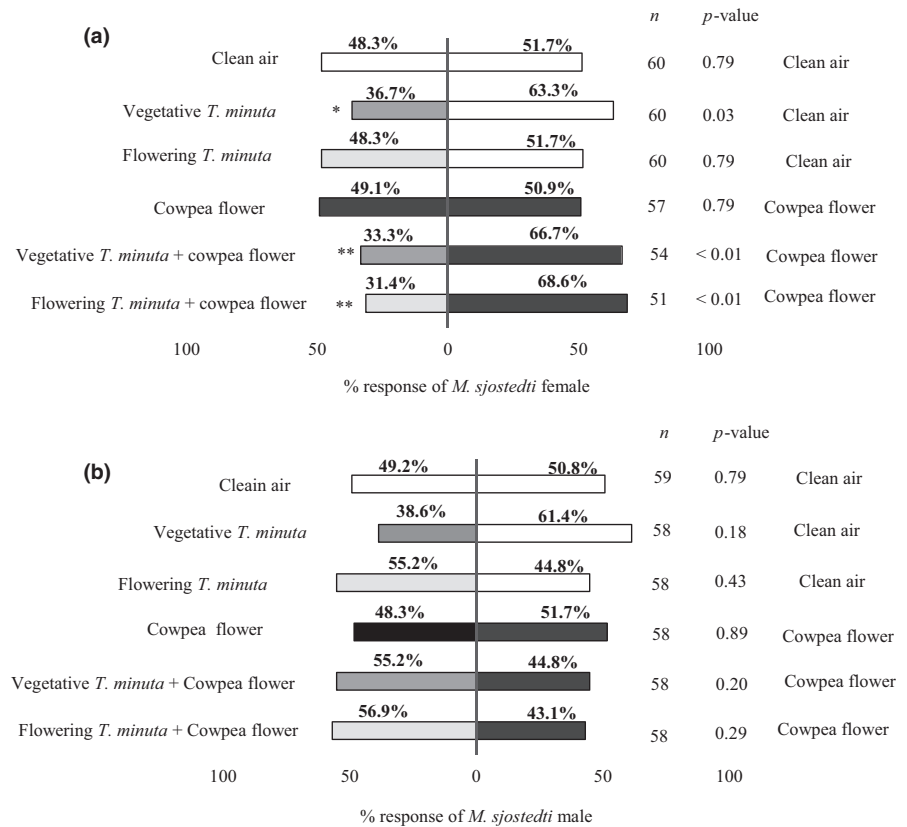
flower combined with fresh cut leaves versus cowpea flower alone ($\chi^2 = 2.28$, $df = 1$, $p = 0.13$) (Figure 1 b).

Vegetative *T. minuta* repelled more females relative to clean air (female: $\chi^2 = 4.26$, $df = 1$, $p = 0.03$) but the males were not repelled ($\chi^2 = 1.72$, $df = 1$, $p = 0.18$) (Figure 2 a). Flowering *T. minuta* had no significant attractive/repellent effect on either females or males. More female *M. sjostedti* were repelled by cowpea flower combined either with vegetative *T. minuta* ($\chi^2 = 7.69$, $df = 1$, $p < 0.01$) or flowering *T. minuta* ($\chi^2 = 7.07$, $df = 1$, $p < 0.01$) compared to cowpea flower alone. A combination of cowpea flower and marigold either at vegetative or flowering stage had no significant effect on males (vegetative: $\chi^2 = 1.61$, $df = 1$, $p = 0.20$, flowering: $\chi^2 = 1.10$, $df = 1$, $p = 0.29$) (Figure 2 b).

3.2 | Analysis of volatiles

Chemical analysis of volatiles collected from the cut leaves of *C. citratus* and *T. minuta* plants revealed 54 compounds (Tables 1 and 2). A total of 30 compounds were identified in fresh and old cut leaves of *C. citratus*: 16 monoterpenoids, 5 sesquiterpenoids, 3 aldehydes, 2 esters, 2 ketones, 1 benzenoid and 1 alcohol (Table 1). Among the compounds, the abundance of (Z)-3-hexenal, (Z)-3-hexenol, (Z)-β-ocimene, (E)-β-ocimene, (E)-isocitral, neral, geraniol and geranial in *C. citratus* was significantly higher in freshly cut leaves than in old cut leaves ($W = 16$, $p = 0.028$). Conversely, the myrcene was higher in old cut leaves than in freshly cut leaves ($W = 16$, $p = 0.028$). Some minor compounds such as (E)-2-hexenal, heptanal, 6,7-epoxy-myrcene, (Z)-isocitral, geranyl acetate, butylated hydroxytoluene

and β-sesquiphellandrene were not detected in old leaves. The PCA explained 85.1% of the total variance, 67.4% of the explanation on the horizontal axis (PC1) and 17.7% on the vertical axis (PC2). The correlation above 0.9 was deemed important, and the compounds (Z)-2-hexenal, (Z)-3-hexenol, (Z)-β-ocimene, (E)-β-ocimene, (E)-isocitral, nerol, neral, geranial, myrcene, α-pinene, limonene, (Z)-linalool oxide, 6,7-epoxymyrcene, 2-undecanone, (E)-caryophyllene, (Z)-β-farnesene, α-humulene, 2-tridecanone and β-sesquiphellandrene were most strongly correlated with the first component and contributed the most to the separation of fresh and old cut leaves of *C. citratus*. However, the values of compounds were not strongly correlated with the second principal component (Table 1).

A total of 24 compounds were identified in the vegetative and flowering *T. minuta*: 9 monoterpenoids, 5 ketones, 5 sesquiterpenoids, 3 esters and 1 aldehyde and 1 alcohol (Table 2). The five most abundant compounds were dihydrotagetone, (Z)-3-hexenyl acetate, limonene, (Z)-β-ocimene and (Z)-tagetone at both phenological stages of *T. minuta*. (Z)-3-Hexenyl acetate was significantly more abundant in the vegetative *T. minuta* than in *T. minuta* with flowers ($W = 1$, $p = 0.057$). Conversely, the emission of (Z)-β-ocimene ($W = 16$, $p = 0.028$), (E)-tagetone ($W = 12$, $p = 0.043$), ethyl 2-methylbutanoate ($W = 15$, $p = 0.059$) and bicyclogermacrene ($W = 15$, $p = 0.057$) was significantly higher in flowering than in vegetative *T. minuta* ($W = 16$, $p = 0.028$). The PC1 on the horizontal axis explained 66.7% of the total variance while PC2 on the vertical axis explained 13.9%. The loadings indicated a greater contribution of α-pinene, camphene, sabinene, myrcene, limonene, (Z)-β-ocimene, (Z)-epoxy-ocimene and (Z)-tagetone to the first component relative

TABLE 1 Abundance (%) of volatile compounds emitted by one gram (1 g) of cut leaves of *C. citratus* at 0 hr and 24 hr (Mean ± SE) and PCA results of loadings of the principal components obtained in the analysis

R.T	Name of compound	Compound class	R.I	Freshly cut leaf of *C. citratus* at 0 hr, n = 4	Old cut leaf of *C. citratus* at 24 hr, n = 4	p-value	PC1	PC2
6.52	(Z)-3-Hexenal	Aldehyde	805	0.25 ± 0.07	0.09 ± 0.06	0.029	0.8314967	0.18270143
8.00	(E)-2-Hexenal	Aldehyde	861	0.00 ± 0.00	–		0.9917567	-0.05930679
8.11	(Z)-3-Hexenol	Alcohol	866	1.20 ± 1.01	0.19 ± 0.11	0.029	0.9979895	-0.02576096
9.20	Heptanal	Aldehyde	909	tr	–		-1,414,517	0.88536609
9.87	α-Pinene[a]	Monoterpenoid	941	0.01 ± 0.00	0.10 ± 0.05	0.771	0.9843458	-0.1166402
10.78	β-Pinene	Monoterpenoid	984	0.00 ± 0.00	0.01 ± 0.01	0.868	-1,907,407	0.8509411
11.09	Myrcene	Monoterpenoid	999	28.68 ± 1.92	41.60 ± 9.06	0.028	0.9795259	0.18594547
11.82	Limonene[a]	Monoterpenoid	1040	0.03 ± 0.03	0.01 ± 0.01	1	0.992147	-0.06205547
11.97	(Z)-β-Ocimene[a]	Monoterpenoid	1048	1.11 ± 0.44	0.54 ± 0.08	0.028	0.9955559	0.01660814
12.16	(E)-β-Ocimene[a]	Monoterpenoid	1059	0.70 ± 0.26	0.50 ± 0.17	0.028	0.9951153	0.0222853
12.61	(Z)-Linalool oxide	Monoterpenoid	1084	0.00 ± 0.00	0.02 ± 0.01	0.868	0.9590568	-0.09403422
12.95	6,7-Epoxymyrcene	Monoterpenoid	1103	0.22 ± 0.20	–		0.9916454	-0.04581753
13.08	Linalool[a]	Monoterpenoid	1111	0.29 ± 0.11	0.26 ± 0.18	0.300	-2,565.975	0.76855528
14.13	(Z)-Isocitral	Monoterpenoid	1177	0.07 ± 0.02	–		-0.2071976	0.76911531
14.40	(E)-Isocitral	Monoterpenoid	1194	0.24 ± 0.05	0.09 ± 0.05	0.029	0.9969823	0.05190482
15.09	Nerol[a]	Monoterpenoid	1227	1.34 ± 0.48	1.93 ± 1.06	0.628	0.9931096	-0.02704885
15.29	Neral[a]	Monoterpenoid	1235	21.62 ± 1.32	15.03 ± 2.17	0.028	0.9886852	0.13468648
15.46	Geraniol[a]	Monoterpenoid	1249	10.99 ± 2.53	7.28 ± 3.80	0.028	0.7556431	0.39497905
15.72	Geranial[a]	Monoterpenoid	1264	31.08 ± 2.63	27.77 ± 3.28	0.028	0.9416674	0.31834731
16.01	2-Undecanone	Ketone	1293	0.65 ± 0.48	0.20 ± 0.13	0.300	0.9951636	-0.03842726
16.27	Carvacrol	Monoterpenoid	1298	–	0.43 ± 0.26		-0.3093366	-0.55452933
16.99	Neryl acetate	Ester	1359	–	0.01 ± 0.01		-0.2001877	-0.23660942
17.26	Geranyl acetate	Ester	1379	0.01 ± 0.01	–		-0.1414517	0.88536609
17.86	(E)-Caryophyllene	Sesquiterpenoid	1444	0.34 ± 0.11	1.04 ± 0.31	0.114	0.9974323	-0.01717904
18.22	(Z)-β-Farnesene	Sesquiterpenoid	1472	0.04 ± 0.07	0.04 ± 0.04	0.877	0.9888328	-0.04905136
18.29	α-Humulene	Sesquiterpenoid	1478	0.03 ± 0.02	0.08 ± 0.06	0.644	0.988139	-0.07661983
18.71	2-Tridecanone	Ketone	1511	0.50 ± 0.20	0.32 ± 0.32	0.183	0.9953826	0.01554321
18.94	Butylated hydroxytoluene	Benzenoid	1531	tr	–		-0.1414517	0.88536609
19.10	β-Sesquiphellandrene	Sesquiterpenoid	1544	0.00 ± 0.00	–		0.9917567	-0.05930679
20.17	Epi-Cedrol	Sesquiterpenoid	1635	–	0.06 ± 0.04		-0.3013739	-0.55113169

Note: Fresh cut leaf of *C. citratus* at 0 hr means the leaves were immediately tested after leaves were cut (olfactory tests were performed between 0 and 1 hr). Old cut leaf of *C. citratus* at 24 hr means the leaves were tested 24 hr after the leaves were cut. *p*-value of comparison of volatile compounds between fresh and old cut leaves of *C. citratus* according to the Mann–Whitney–Wilcoxon test.

Abbreviations: –, not detected; hr, hours; PC1, first principal component; PC2, second principal component; R.I, retention indices calculated relative to *n*-alkanes C8-C30 on HP-5MS column; R.T, retention time; tr, trace < 0.005.

[a]compounds identified by library data and authentic standards.

TABLE 2 Abundance (%) of volatile compounds emitted by the vegetative and flowering *Tagetes minuta* (Mean ± SE) and PCA results of loadings of the principal components obtained in the analysis

R.T	Compound name	Compound class	R.I	Vegetative *T. minuta*, n = 4	Flowering *T. minuta*, n = 4	p-value	PC1	PC2
6.52	(Z)-3-Hexenal	Aldehyde	805	0.65 ± 0.39	0.10 ± 0.06	0.300	-0.24256284	-0.87185715
7.98	Ethyl 2-methylbutanoate	Ester	861	0.28 ± 0.05	0.51 ± 0.14	0.059	0.84565218	0.0953021
8.11	(Z)-3-Hexenol	Alcohol	866	0.47 ± 0.45	–		-0.25372832	-0.77986057
9.87	α-Pinene[a]	Monoterpenoid	941	0.15 ± 0.09	0.21 ± 0.06	0.114	0.90907226	0.27574235
10.19	Camphene	Monoterpenoid	956	0.18 ± 0.06	0.23 ± 0.04	0.485	0.96805186	-0.10177755
10.72	Sabinene	Monoterpenoid	981	1.60 ± 0.64	2.39 ± 0.67	0.200	0.96511014	0.1376464
11.09	Myrcene	Monoterpenoid	999	0.07 ± 0.04	0.37 ± 0.03	0.110	0.97032444	0.15011675
11.4	(Z)-3-Hexenyl acetate[a]	Ester	1016	25.56 ± 4.96	1.95 ± 0.60	0.057	-0.28488187	-0.72732406
11.82	Limonene[a]	Monoterpenoid	1040	10.36 ± 3.64	14.74 ± 3.44	0.200	0.98214275	0.07698571
11.97	(Z)-β-Ocimene[a]	Monoterpenoid	1048	7.73 ± 2.03	16.32 ± 0.53	0.028	0.98823445	0.1082103
12.23	Dihydrotagetone[a]	Ketone	1063	34.63 ± 8.34	30.91 ± 1.07	0.200	0.89761887	0.0536998
12.95	6,7-Epoxymyrcene	Monoterpenoid	1103	–	0.01 ± 0.01		0.40812653	-0.43967586
13.08	Linalool[a]	Monoterpenoid	1111	0.05 ± 0.03	0.03 ± 0.01	0.644	0.88280274	-0.31149095
13.62	(Z)-Epoxy-ocimene	Monoterpenoid	1145	0.03 ± 0.02	0.20 ± 0.05	0.110	0.92815204	-0.16245713
13.79	(E)-Tagetone	Ketone	1156	0.08 ± 0.08	0.90 ± 0.57	0.043	0.56247659	-0.1113343
13.92	(Z)-Tagetone	Ketone	1164	14.25 ± 4.48	18.52 ± 3.19	0.342	0.94573447	-0.20985552
14.66	Methyl salicylate	Ester	1212	tr	0.00 ± 0.00	1	0.3914376	-0.54727624
15.13	(Z)-Ocimenone	Ketone	1244	0.07 ± 0.07	0.18 ± 0.10	0.408	0.95869803	-0.21909826
15.26	Car-3-en-2-one	Ketone	1253	1.66 ± 1.66	7.65 ± 0.68	0.103	0.96110602	-0.22915292
17.26	α-Copaene	Sesquiterpenoid	1397	tr	0.00 ± 0.00	0.538	-0.06868269	-0.67759745
17.86	(E)-Caryophyllene	Sesquiterpenoid	1444	0.62 ± 0.10	1.35 ± 0.13	0.200	0.97658595	0.057171
18.29	α-Humulene	Sesquiterpenoid	1478	0.04 ± 0.03	0.15 ± 0.03	0.110	0.9719888	-0.13232112
18.63	Germacrene D	Sesquiterpenoid	1505	0.13 ± 0.05	0.60 ± 0.21	0.342	0.93642974	0.08027518
18.83	Bicyclogermacrene	Sesquiterpenoid	1521	0.50 ± 0.20	2.30 ± 0.43	0.057	0.95767035	0.10791004

Note: p-value of comparison of volatile compounds between vegetative and flowering stages of *T. minuta*, according to the Mann–Whitney–Wilcoxon test.

Abbreviations: –, not detected; PC1, first principal component; PC2, second principal component; R.I, retention indices calculated relative to n-alkanes C8-C30 on HP-5MS column; R.T, retention time; tr, trace < 0.005.

[a]compounds identified by library data and authentic standards.

(a)

	n	p-value	
Control — 49.2% / 50.8%	59	0.89	Control
Myrcene (1%) — 54.5% / 45.5%	44	0.54	Control
Nerol (1%) — 40.4% / 59.6%	52	0.16	Control
Citral (1%) ** — 25.5% / 74.5%	47	< 0.01	Control
Geraniol (1%) — 55.6% / 44.4%	54	0.41	Control
Blend (1%) — 44.2% / 55.8%	52	0.40	Control

100   50   0   50   100
% response of *M. sjostedti* female

(b)

	n	p-value	
Cowpea flower — 49.1% / 50.9%	59	0.89	Cowpea flower
Myrcene (1%) + Cowpea flower * — 67.3% / 32.7%	49	0.01	Cowpea flower
Nerol (1%) + Cowpea flower — 53% / 47%	49	0.66	Cowpea flower
Citral (1%) + Cowpea flower — 55.3% / 44.7%	57	0.46	Cowpea flower
Geraniol (1%) + Cowpea flower — 59.6% / 40.4%	52	0.16	Cowpea flower
Blend (1%) + Cowpea flower — 47.2% / 52.8%	53	0.68	Cowpea flower

100   50   0   50   100
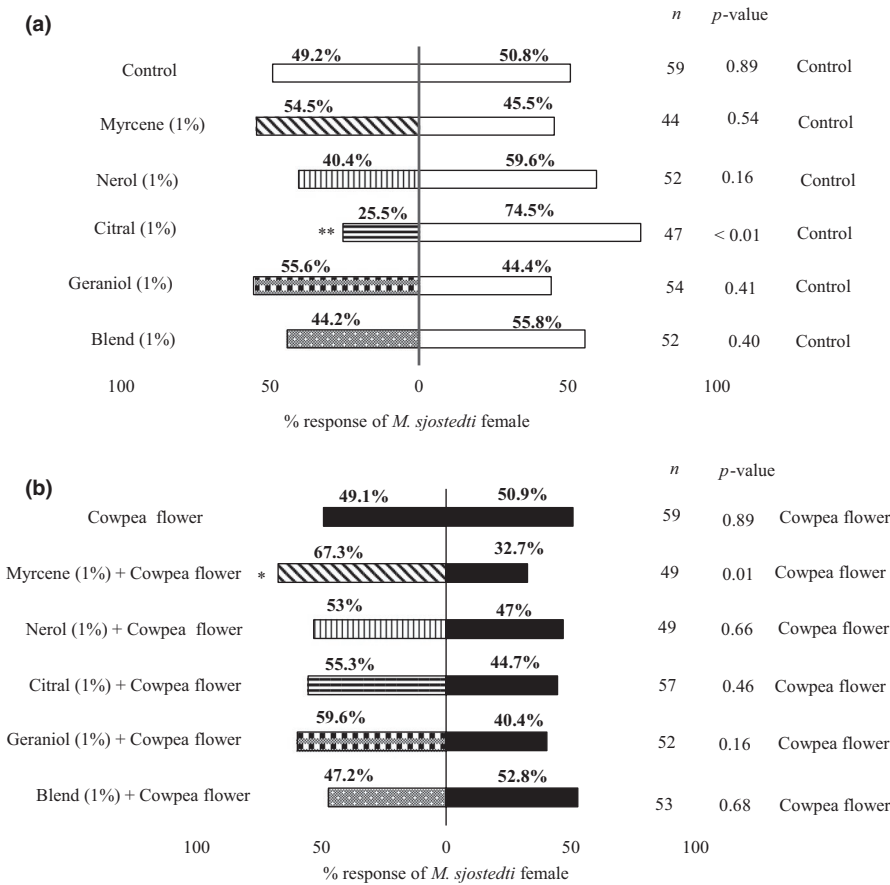% response of *M. sjostedti* female

FIGURE 3 Percentage (%) responses of *M. sjostedti* female to major compounds of freshly cut leaves of *C. citratus* in a Y-tube olfactometer. (a) Each compound or blend (citral, myrcene, geraniol, nerol) versus control (solvent) and (b) each compound or blend (citral, myrcene, geraniol, nerol) + cowpea flower versus cowpea flower alone. The compounds tested in the Y-tube olfactometer were diluted in dichloromethane at a concentration of 1%. *n* = total number of insects which responded per treatment. Significance levels of χ^2 tests are indicated by *$p < 0.05$, **$p < 0.01$, (χ^2 goodness-of-fit test)

(a)

	n	p-value	
Control — 49.2% / 50.8%	59	0.89	Control
(+) - (R) -Limonene (1%) — 47.1% / 52.9%	51	0.67	Control
(+) - (S) -Limonene (1%) — 40% / 60%	50	0.15	Control
Ocimene (1%) — 42% / 58%	50	0.25	Control
(Z)-3-Hexenyl acetate (1%) — 40% / 60%	50	0.15	Control
Dihydrotagetone (1%) ** — 70.5% / 29.5%	44	< 0.01	Control
Blend (1%) ** — 31.2% / 68.8%	48	< 0.01	Control

100   50   0   50   100
% response of *M. sjostedti* female

(b)

	n	p-value	
Cowpea flower — 49.1% / 50.9%	57	0.89	Cowpea flower
Limonene (1%) + Cowpea flower — 47.1% / 52.9%	51	0.67	Cowpea flower
Ocimene (1%) + Cowpea flower — 56.4% / 43.6%	55	0.34	Cowpea flower
(Z)-3-Hexenyl acetate (1%) + Cowpea flower — 48.1% / 51.9%	54	0.7	Cowpea flower
Dihydrotagetone (1%) + Cowpea flower — 46.9% / 53.1%	49	0.66	Cowpea flower
Blend (1%) + Cowpea flower * — 32.7% / 67.3%	55	0.01	Cowpea flower

100   50   0   50   100
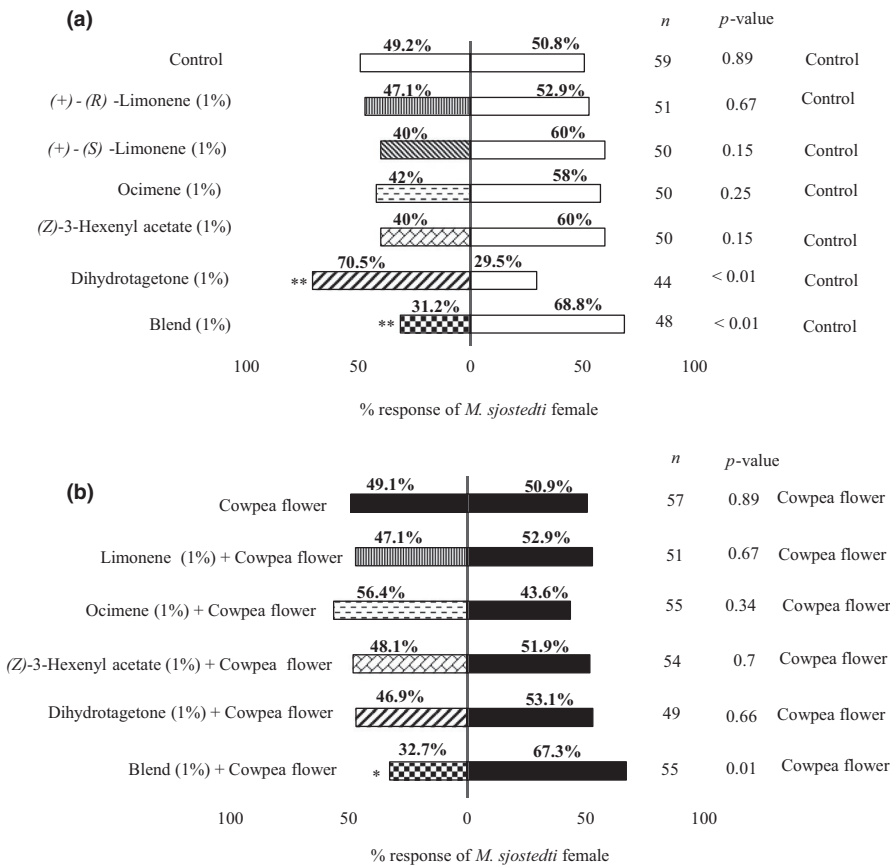% response of *M. sjostedti* female

FIGURE 4 Percentage (%) responses of female *M. sjostedti* to major compounds of vegetative *T. minuta* in a Y-tube olfactometer. (a) Each compound or blend (dihydrotagetone, (Z)-3-hexenyl acetate, limonene and (Z)-β-ocimene) versus control (solvent) and (b) each compound or blend (dihydrotagetone, (Z)-3-hexenyl acetate, limonene and (Z)-β-ocimene) + cowpea flower versus cowpea flower alone. The compounds tested in the Y-tube olfactometer were diluted in dichloromethane at a concentration of 1%. *n* = total number of insects which responded per treatment. Significance levels of chi-square tests are indicated by *$p < 0.05$, **$p < 0.01$, (χ^2 goodness-of-fit test)

to the second component, thus separating the vegetative and flowering stages of *T. minuta* (Table 2).

3.3 | Behavioural response of *M. sjostedti* to synthetic standards

Among the five major monoterpenoids (geranial, myrcene, neral, geraniol and nerol) identified in the cut leaves of *C. citratus*, only citral showed a repellent effect on female *M. sjostedti* relative to control (χ^2 = 7.36, *df* = 1, *p* < 0.01) (Figure 3a). The blend of these five monoterpenoids was not repellent (χ^2 = 0.69, *df* = 1, *p* = 0.40). Interestingly, the combination of myrcene and cowpea flower was preferred by female *M. sjostedti* to cowpea flower alone (χ^2 = 5.89, *df* = 1, *p* = 0.01). Female *M. sjostedti* could not distinguish cowpea flower combined with either nerol, citral, geraniol or a blend of these compounds from cowpea flower alone (Figure 3b).

Among the four major compounds (dihydrotagetone, (Z)-3-hexenyl acetate, limonene and (Z)-β-ocimene) of *T. minuta* tested, dihydrotagetone was the only one that elicited significant behavioural response. Surprisingly, dihydrotagetone was an attractant to female *M. sjostedti* relative to control (χ^2 = 7.36, *df* = 1, *p* < 0.01) (Figure 4a). However, female *M. sjostedti* were repelled by the blend of the four compounds relative to clean air (χ^2 = 6.75, *df* = 1, *p* < 0.01) or repelled by the combination of blend + cowpea flower relative to cowpea flower alone (χ^2 = 6.56, *df* = 1, *p* = 0.01) (Figure 4b).

4 | DISCUSSION

Results from the current study show that volatiles from *T. minuta* and from freshly cut leaves of *C. citratus* were repellent to female *M. sjostedti*. Evidence of the repellent effect of extracts and essential oils from both plants on several insects, including thrips, has been reported in previous work (Abtew et al., 2015; Cornelius & Wycliffe, 2016). In our study, male and female thrips were repelled by the volatiles from freshly cut leaves, but old cut leaves were not repellent to either males or females. In a previous study, Abtew et al. (2015) reported the repellent effect of *C. citratus* extract on female *M. sjostedti* in a vertical olfactometer. However, the authors did not test the repellent effect of *C. citratus* extract on male *M. sjostedti*. In the present study, the male *M. sjosedti* were not repelled by the combination of cowpea flower and freshly cut leaves of *C. citratus*; therefore, an experiment on the combination with old cut leaves of *C. citratus* was not conducted. In addition, the major compounds from *C. citratus* were not tested alone or combined with cowpea flower on the male *M. sjostedti*. In our previous study, among four cowpea cultivars tested on the male and female *M. sjostedti*, only the flowers of cowpea var. Ken Kunde 1 were attractive to the female thrips while the males were not attracted to the cowpea volatiles (Diabate et al., 2019). The cowpea volatiles did not attract the males, and thus, the repellent or masking effect of major compounds of *C. citratus* with cowpea flower was not tested on the males.

PCA analysis indicated a difference in the profiles of volatile emissions between the fresh and old cut leaves of *C. citratus*. The absence of response in both male and female *M. sjostedti* to the old cut leaves could be due to the decrease in the abundance of (Z)-3-hexenal, (Z)-3-hexenol, (Z)-β-ocimene, (E)-β-ocimene, (E)-isocitral, neral, geraniol and geranial, and/or the absence of certain volatile compounds such as (E)-2-hexenal, heptanal, 6,7-epoxymyrcene, (Z) isocitral, geranyl acetate, butylated hydroxytoluene and β- sesquiphellandrene in the leaves. In the olfactory tests, citral (neral + geranial) was repellent to females compared to clean air. These results suggest that citral, and particularly neral, is involved in the repellence of *C. citratus* leaves to *M. sjostedti*. Abtew (2015) reported that citral was a good repellent for *M. sjostedti* larvae. However, in combination with cowpea flower var. Ken Kunde 1, citral was not repellent. The background plant volatiles can affect the behavioural response of thrips to the compound (Koschier, Nielsen, Spangl, Davidson, & Teulon, 2017). For example, salicylaldehyde, a repellent compound of western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), elicited a neutral response in *F. occidentalis* when it was combined with flowering chrysanthemums, *Chrysanthemum morifolium* Ramat (host plant) versus flowering chrysanthemums without compound in Y-tube olfactory tests (Koschier et al., 2017). The neutral response of *M. sjostedti* to the old leaves of *C. citratus* may be due to the decline in amounts of neral in the old leaves. On the one hand, the abundance of myrcene in the old cut leaves may explain the decline in repellence of *M. sjostedti*. In fact, in our assays, myrcene tested alone was not repellent to females. On the other hand, the blend of myrcene and cowpea flower volatiles attracted females. The abundance of myrcene in the volatiles may contribute to the attraction of *M. sjostedti* and could indicate a suitable resource for feeding or egg-laying. Additionally, myrcene appears to enhance the attraction of cowpea flowers for female *M. sjostedti*. Alone, the volatiles emitted by a non-host plant may have no effect, but in combination, they may enhance the response of insects to the host plant. The background odours may (a) mask the odour source, (b) enhance the insect's responses to the odour source or (c) have no effect on the odour source (Schröder & Hilker, 2008).

The absence of a response by female *M. sjostedti* to the blend of major compounds of *C. citratus* suggests that minor compounds may also contribute to the repellent effect of the plant volatiles. For example, (E)-2-hexenal was repellent to *M. sjostedti* females in olfactory tests (Diabate et al., 2019). According to Hummelbrunner and Isman (2001) the low concentrations of minor compounds present in plant volatiles may act as synergists, enhancing the effectiveness of the major compounds through a variety of mechanisms. The effect of interactions between minor and major compounds on the behavioural response of *M. sjostedti* needs further investigation.

Female *M. sjostedti* were repelled by cowpea flower combined with freshly cut leaves of *C. citratus* relative to the cowpea flower alone. These results suggest that the volatile emitted by fresh cut leaves of *C. citratus* reduces the attractiveness of the cowpea flower to female *M. sjostedti*. The efficacy of volatiles from *C. citratus* in controlling insect pests has been demonstrated in the field. For

example, the volatiles from *C. citratus* intercropped with eggplant, *Solanum melongena* L, reduced the infestation of moth *Leucinodes orbonalis* Guenee in eggplant plants (Calumpang, Bayot, Vargas, Ebuenga, & Gonzales, 2013).

In our study, female *M. sjostedti* were repelled by vegetative *T. minuta* but not the flowering *T. minuta* while males were not attracted/repelled by *T. minuta* volatiles. The differential responses of male and female *Ceratitis capitata* Wiedemann insects to the volatiles from *T. minuta* have also been reported by López et al. (2011): in a Y-tube olfactometer, *T. minuta* oil was attractive to *C. capitata* males but females avoided the oil. Male and female thrips sometimes show different levels of response to plant odours (Cao et al., 2017). For instance, in olfactory tests of *F. occidentalis* to odours from six vegetable plants (cabbage, lettuce, cucumber, eggplant, celery and garlic), females were attracted to the volatiles from undamaged or damaged cabbage, lettuce, cucumber and eggplant, while the males were attracted to volatiles from undamaged lettuce and eggplant, and damaged cucumber (Cao, Zhi, Cong, & Margolies, 2014). In our study, female *M. sjostedti* seemed to be more susceptible to the plant volatiles than the males. On the other hand, the phenological stage may also affect the attraction of female *M. sjostedti* to *T. minuta* plants. PCA showed that the volatile profile of *T. minuta* differed in the vegetative and flowering stages. The compounds (*Z*)-*β*-ocimene, (*E*)-tagetone, ethyl 2-methylbutanoate and bicyclogermacrene were abundant during flowering while (*Z*)-3-hexenyl acetate was abundant in the vegetative stage of *T. minuta*. (*Z*)-3-Hexenyl acetate is one of the green leaf volatiles (GLVs) which are typically released by plants immediately after wounding or stress (Scala, Allmann, Mirabella, Haring, & Schuurink, 2013). (*Z*)-*β*-Ocimene and (*Z*)-3-hexenyl acetate were two of the major compounds of *T. minuta*, and their individual assays in a Y-tube olfactometer did not elicit a behavioural response from the female *M. sjostedti* compared to clean air. However, when the four major compounds ((*Z*)-*β*-ocimene, (*Z*)-3-hexenyl acetate, dihydrotagetone and limonene) were combined in the same ratio of volatiles released by the vegetative stage of *T. minuta*, female *M. sjostedti* were repelled by the synthetic blend. The repellent effect of the vegetative *T. minuta* may be due to the synergistic effect of four major compounds. However, (*Z*)-tagetone, a major compound of *T. minuta*, was not commercially available, so it was not included in the bioassays. Insects use the appropriate blend composition with species-specific ratios of different compounds to recognize a host plant (Bruce, Wadhams, & Woodcock, 2005; Webster, 2012). Webster, Bruce, Pickett, and Hardie (2010) showed that the blend of host plant odours was attractive to the black bean aphid, *Aphis fabae* Scopoli, whereas individual volatile constituents repelled them. Further investigation needs to be performed for more clarification of synergistic effects (binary/ternary/quaternary and other combinations) of the compounds.

The combination of flowering *T. minuta* with open cowpea flowers was repellent to female *M. sjostedti*. This suggests that flowering *T. minuta* volatiles interfere with *M. sjostedti* orientation towards cowpea flowers. Dardouri, Gautier, Costagliola, and

Gomez (2017) showed that the flowering stage of *Tagetes patula* L. had no repellent effect on *Myzus persicae* Sulzer aphids. However, when the odours of pepper and *T. patula* plants were mixed and compared to pepper odours alone, aphids showed a significant preference for the host pepper plant. Hence, a mixture of volatiles from cowpea flower and flowering *T. minuta* may affect the attraction of female *M. sjostedti*.

Dihydrotagetone was the main compound identified in the volatiles released by the vegetative and flowering stages of *T. minuta*. Tested individually, dihydrotagetone elicited an attractive response from female *M. sjostedti*. These results indicate that this compound was not involved in the repellence of the vegetative stage of *T. minuta* to female *M. sjostedti*. We can assume that the attraction of dihydrotagetone was masked by the other major compounds in the vegetative stage. Numerous studies have documented the attraction of thrips to several compounds from floral scents (Koschier, 2006). For example, *p*-anisaldehyde, a common flower volatile component, attracts *F. occidentalis* (Koschier et al., 2000). Dihydrotagetone was present at different concentrations in flowering and vegetative stages of *T. minuta*. In-depth investigations of the mechanism involved in the repellent effect of *M. sjostedti* in different concentrations could advance our understanding of thrip behaviour.

In the present study, after observing the repellent effect of both plants to female thrips, we expected to record a repellent effect of the major compounds in these herbal plants, tested individually or in a blend in the natural ratio. However, we found that citral alone and the blend of major compounds of vegetative *T. minuta* alone or in combination with cowpea flower (attractant) were repellent to female *M. sjostedti*. Surprisingly, we found new attractant compounds (dihydrotagetone, myrcene) for females of *M. sjostedti*.

To conclude, this study has shown that volatiles from freshly cut leaves of *C. citratus* repelled males and females of *M. sjostedti* but old cut leaves did not. The freshly cut leaves of *C. citratus* combined with cowpea flower repelled female *M. sjostedti*. Female *M. sjostedti* were repelled by the volatiles from the vegetative stage of *T. minuta* and repelled by cowpea flower combined with either vegetative or flowering *T. minuta*. Conversely, the behavioural response of males was not affected by any odour from the two phenological stages of *T. minuta*. Citral, a major compound of *C. citratus*, and the blend of major compounds (dihydrotagetone, (*Z*)-3-hexenyl acetate, limonene and (*Z*)-*β*-ocimene) from vegetative *T. minuta* were repellent to females. Dihydrotagetone alone and myrcene combined with cowpea flower attracted the female *M. sjostedti*. Finally, this study identified and characterized two repellent companion plants for *M. sjostedti* that can be used in pest management. After further investigation, the blend of major compounds of *T. minuta* could be used as a repellent with a diffuser in the field. *T. minuta* plant and the freshly cut leaves of *C. citratus* could be used to control *M. sjostedti* in the field. Further studies are needed to investigate the repellent effect of both plants and their arrangement in intercropping. The exact repellence duration of the cut leaves on *M. sjostedti* should be investigated in a further study, because this effect may pose a major obstacle to the practical application of *C. citratus*.

CONFLICT OF INTEREST

No conflict of interest to declare.

AUTHOR CONTRIBUTION

Author 2 and author 7 conceived the research. Author 1 conducted experiments. Author 1 and author 2 contributed material. Author 1 and author 2 analysed data and conducted statistical analyses. Author 1, author 2, author 3, author 4, author 5, author 6 and author 7 wrote the manuscript. Author 4 secured funding. All authors read and approved the manuscript.

DATA AVAILABILITY

All data are available from CIRAD Dataverse, v1 (https://doi.org/10.18167/DVN1/72WX4Q).

ORCID

Seydou Diabate https://orcid.org/0000-0001-6658-1547

Komi K. M. Fiaboe https://orcid.org/0000-0001-5113-2159

Sevgan Subramanian https://orcid.org/0000-0003-4447-0744

REFERENCES

Abtew, A. B. (2015). The behavior, ecology and control of legume flower thrips, *Megalurothrips sjostedti* (Trybom) in cowpea *Vigna unguiculata* (L.) towards the development of an integrated pest management (IPM) program in Kenya. Ph.D dissertation, Montpellier SupAgro (France) and University of Catania (Italy), p. 141. http://34.250.91.188:8080/xmlui/handle/123456789/265

Abtew, A., Niassy, S., Affognon, H., Subramanian, S., Kreiter, S., Garzia, G. T., & Martin, T. (2016). Farmers' knowledge and perception of grain legume pests and their management in the Eastern province of Kenya. *Crop Protection*, 87, 90–97. https://doi.org/10.1016/j.cropro.2016.04.024

Abtew, A., Subramanian, S., Cheseto, X., Kreiter, S., Garzia, G. T., & Martin, T. (2015). Repellency of plant extracts against the legume flower thrips *Megalurothrips sjostedti* (Thysanoptera: Thripidae). *Insects*, 6, 608–625. https://doi.org/10.3390/insects6030608

Adams, R. P. (1995). *Identification of essential oil components by gas chromatography/mass spectrometry*. Carol Stream, IL: Allured.

Basedow, T., Hua, L., & Aggarwal, N. (2006). The infestation of *Vicia faba* L. (Fabaceae) by *Aphis fabae* (Scop.) (Homoptera: Aphididae) under the influence of Lamiaceae (*Ocimum basilicum* L. and *Satureja hortensis* L.). *Journal of Pest Science*, 79, 149–154. https://doi.org/10.1007/s10340-006-0128-7

Bruce, T. J. A., Wadhams, L. J., & Woodcock, C. M. (2005). Insect host location: A volatile situation. *Trends in Plant Science*, 10, 269–274. https://doi.org/10.1016/j.tplants.2005.04.003

Calumpang, S. M. F., Bayot, R. G., Vargas, D. G., Ebuenga, D. M., & Gonzales, P. G. (2013). Impact of intercropping lemon grass (*Cymbopogon citratus* Stapf.) on infestation of eggplant fruit and shoot borer (*Leucinodes orbonalis* Guenee) in Eggplant (*Solanum melongena* L.). *Silliman Journal*, 54, 114–130.

Cao, Y., Zhi, J., Cong, C., & Margolies, D. C. (2014). Olfactory cues used in host selection by *Frankliniella occidentalis* (Thysanoptera: Thripidae) in relation to host suitability. *Journal of Insect Behavior*, 27, 41–56. https://doi.org/10.1007/s10905-013-9405-5

Cao, Y., Zhi, J., Li, C., Zhang, R., Wang, C., Shang, B., & Gao, Y. (2017). Behavioral responses of *Frankliniella occidentalis* to floral volatiles combined with different background visual cues. *Arthropod Plant Interactions*, 12, 31–39. https://doi.org/10.1007/s11829-017-9549-x

Cornelius, W. W., & Wycliffe, W. (2016). Tagetes (*Tagetes minuta*) oils. In V. R. Preedy (Ed.), *essential oils in food preservation, flavor and safety* (pp. 791–802). San Diego, CAAcademic Press.

Dardouri, T., Gautier, H., Costagliola, G., & Gomez, L. (2017). How French marigold (*Tagetes patula* L.) volatiles can affect the performance of green peach aphid. *Integrated Protection of Fruit Crops*, 123, 71 – 78.

de Bon, H., Huat, J., Parrot, L., Sinzogan, A., Martin, T., Malézieux, E., & Vayssières, J. F. (2014). Pesticide risks from fruit and vegetable pest management by small farmers in sub-Saharan Africa. *A Review. Agronomy for Sustainable Development*, 34(4), 723–736. https://doi.org/10.1007/s13593-014-0216-7

Deletre, E., Schatz, B., Bourguet, D., Chandre, F., Williams, L., Ratnadass, A., & Martin, T. (2016). Prospects for repellent in pest control: Current developments and future challenges. *Chemoecology*, 26, 127–142. https://doi.org/10.1007/s00049-016-0214-0

Diabate, S., Deletre, E., Murungi, L. K., Fiaboe, K. K. M., Subramanian, S., Wesonga, J., & Martin, T. (2019). Behavioural responses of bean flower thrips (*Megalurothrips sjostedti*) to vegetative and floral volatiles from different cowpea cultivars. *Chemoecology*, 29, 73–88. https://doi.org/10.1007/s00049-019-00278-0

Dray, S., & Dufour, A. B. (2007). The ADE4 package: Implementing the duality diagram for ecologists. *Journal of Statistical Software*, 22, 1–20. https://doi.org/10.18637/jss.v022.i04

Ekesi, S., Maniania, N. K., & Onu, I. (1999). Effects of temperature and photoperiod on development and oviposition of the legume flower thrips, *Megalurothrips sjostedti*. *Entomologia Experimentalis Et Applicata*, 93, 149–155. https://doi.org/10.1046/j.1570-7458.1999.00573.x

FAOSTAT (2016). Crops. http://www.fao.org/faostat/en/#data/QC. Accessed 19th October 2018.

Hummelbrunner, L. A., & Isman, M. B. (2001). Acute, sublethal, anti-feedant, and synergistic effects of monoterpenoid essential oil compounds on the tobacco cutworm, *Spodoptera litura* (Lep., Noctuidae). *Journal of Agricultural and Food Chemistry*, 49, 715–720. https://doi.org/10.1021/jf000749t

Koschier, E. H. (2006). Chapter 10 Plant allelochemicals in thrips control strategies. *Advances in Phytomedicine*, 3, 221–249. https://doi.org/10.1016/S1572-557X(06)03010-8.

Koschier, E. H., De Kogel, W. J., & Visser, J. H. (2000). Assessing the attractiveness of volatile plant compounds to western flower thrips *Frankliniella occidentalis*. *Journal of Chemical Ecology*, 26, 2643–2655.

Koschier, E. H., Nielsen, M. C., Spangl, B., Davidson, M. M., & Teulon, D. A. J. (2017). The effect of background plant odours on the behavioural responses of *Frankliniella occidentalis* to attractive or repellent

compounds in a Y-tube olfactometer. *Entomologia Experimentalis Et Applicata*, *163*, 160–169, https://doi.org/10.1111/eea.12566

López, S. B., López, M. L., Aragón, L. M., Tereschuk, M. L., Slanis, A. C., Feresin, G. E., ... Tapia, A. A. (2011). Composition and anti-insect activity of essential oils from *Tagetes* L. species (Asteraceae, Helenieae) on *Ceratitis capitata* Wiedemann and *Triatoma infestans* Klug. *Journal of Agricultural and Food Chemistry*, *59*, 5286–5292. https://doi.org/10.1021/jf104966b

Moreau, T. L., Warman, P. R., & Hoyle, J. (2006). An evaluation of companion planting and botanical extracts as alternative pest controls for the colorado potato beetle. *Biological Agriculture and Horticulture*, *23*, 351–370. https://doi.org/10.1080/01448765.2006.9755336

Nerio, L. S., Olivero-Verbel, J., & Stashenko, E. (2010). Repellent activity of essential oils: A review. *Bioresource Technology*, *101*, 372–378. https://doi.org/10.1016/j.biortech.2009.07.048

NIST (2008). *Mass spectral library (NIST/EPA/NIH)*. Gaithersburg, USA: National Institute of Standards and Technology.

Nyasani, J. O., Meyhöfer, R., Subramanian, S., & Poehling, H. M. (2013). Seasonal abundance of western flower thrips and its natural enemies in different French bean agroecosystems in Kenya. *Journal of Pest Science*, *86*, 515–523. https://doi.org/10.1007/s10340-013-0491-0

OECD (2015). Consensus document on the biology of cowpea (*Vigna unguiculata* (L.) Walp.). Series on harmonisation of regulatory oversight in biotechnology No. 60.

Parker, J. E., Snyder, W. E., Hamilton, G. C., & Rodriguez-Saona, C. (2013). Companion planting and insect pest control. In S. Soloneski, & M. Larramendy (Eds.), *Weed and Pest Control – Conventional and New Challenges* (pp. 1–30). Rijeka, Croatia: InTech.

Parolin, P., Bresch, C., Desneux, N., Brun, R., Bout, A., Boll, R., & Poncet, C. (2012). Secondary plants used in biological control: A review. *International Journal of Pest Management*, *58*, 91–100. https://doi.org/10.1080/09670874.2012.659229

Popelka, J. C., Gollasch, S., Moore, A., Molvig, L., & Higgins, T. J. V. (2006). Genetic transformation of cowpea (*Vigna unguiculata* L.) and stable transmission of the transgenes to progeny. *Plant Cell Reports*, *25*, 304–312. https://doi.org/10.1007/s00299-005-0053-x

R Core Team (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Sani, I., & Umar, K. M. (2017). Biology and management of legume flower thrips (*Megalurothrips sjostedti*) (Thysanoptera: Thripidae), a major insect pest of cowpea: A review. *Annals of Experimental Biology*, *5*, 14–17.

Scala, A., Allmann, S., Mirabella, R., Haring, M. A., & Schuurink, R. C. (2013). Green leaf volatiles: A plant's multifunctional weapon against herbivores and pathogens. *International Journal of Molecular Sciences*, *14*, 17781–17811. https://doi.org/10.3390/ijms140917781

Schader, C., Zaller, J. G., & Köpke, U. (2005). Cotton-basil intercropping: Effects on pests, yields and economical parameters in an organic field in Fayoum. *Egypt. Biological Agriculture and Horticulture*, *23*, 59–72. https://doi.org/10.1080/01448765.2005.9755308

Schröder, R., & Hilker, M. (2008). The relevance of background odor in resource location by insects: A behavioral approach. *BioScience*, *58*, 308–316. https://doi.org/10.1641/B580406

Singh, B. B., Ajeigbe, H. A., Tarawali, S. A., Fernandez-Rivera, S., & Abubakar, M. (2003). Improving the production and utilization of cowpea as food and fodder. *Field Crops Research*, *84*, 169–177. https://doi.org/10.1016/S0378-4290(03)00148-5

Singh, P., Krishna, A., Kumar, V., Krishna, S., Singh, K., Gupta, M., & Singh, S. (2015). Chemistry and biology of industrial crop *Tagetes* species: A review. *Journal of Essential Oil Research*, *28*, 1 – 14. https://doi.org/10.1080/10412905.2015.1076740

Togola, A., Boukar, O., Belko, N., Chamarthi, S. K., Fatokun, C., Tamo, M., & Oigiangbe, N. (2017). Host plant resistance to insect pests of cowpea (*Vigna unguiculata* L. Walp.): Achievements and future prospects. *Euphytica*, *213*, 1–16. https://doi.org/10.1007/s10681-017-2030-1

USEPA (2012). Biopesticides registration action document -. Tagetes Oil - PC Code, 176602.

Webster, B. (2012). The role of olfaction in aphid host location. *Physiological Entomology*, *37*, 10–18. https://doi.org/10.1111/j.1365-3032.2011.00791.x

Webster, B., Bruce, T., Dufour, S., Birkemeyer, C., Birkett, M., Hardie, J., & Pickett, J. (2008). Identification of volatile compounds used in host location by the black bean aphid, *Aphis fabae*. *Journal of Chemical Ecology*, *34*, 1153–1161. https://doi.org/10.1007/s10886-008-9510-7

Webster, B., Bruce, T., Pickett, J., & Hardie, J. (2010). Volatiles functioning as host cues in a blend become nonhost cues when presented alone to the black bean aphid. *Animal Behaviour*, *79*, 451–457. https://doi.org/10.1016/j.anbehav.2009.11.028

Webster, B., & Cardé, R. T. (2016). Use of habitat odour by host-seeking insects. *Biological Reviews*, *92*, 1241–1249. https://doi.org/10.1111/brv.12281