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Momordica cochinchinensis (Cucurbitaceae) leaf volatiles: semiochemicals for host location by the insect pest, *Aulacophora foveicollis* (Coleoptera: Chrysomelidae)

Abhishek Mukherjee · Nupur Sarkar · Anandamay Barik

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Abstract *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae) and *Epilachna dodecastigma* (Wied.) (Coleoptera: Coccinellidae) are important herbivore pests of *Momordica cochinchinensis* Spreng (Cucurbitaceae).The volatile organic compound (VOC) profile from undamaged and mechanically damaged plants, and from plants 24 h and 120 h following continuous feeding of adult female *A. foveicollis* and *E. dodecastigma*, was identified and quantified by GC-MS and GC-FID analyses. Twenty-two compounds were identified in volatiles of undamaged plants and in 24-h and 120-h post-insect-feeding plant volatiles; whereas 21 components were detected in volatiles of mechanically damaged plants. With the exception of four compounds, 1-heptanol, 3-octanone, acetophenone and nerolidol, the emissions of all other compounds were significantly increased following insect attack. In all plants, phytol was predominant, followed by geranyl linalool and linalool. Only 2-hexanol was unique to mechanically damaged plants, and 1-octen-3-ol and farnesyl acetone were detected in volatiles of undamaged and insect-damaged plants, but not in volatiles of mechanically damaged plants. However, none of these volatile components, when tested individually, showed attraction to *A. foveicollis* in Y-shaped glass tube olfactometer bioassays. *Aulacophora foveicollis* elicited significant preference for the whole volatile blends from insect-damaged plants compared to those of undamaged plants, and volatiles from 120-h post-*E. dodecastigma* feeding plants were more attractive to *A. foveicollis* compared to those from conspecifically damaged plants. Furthermore, the finding that *A. foveicollis* responds to individual synthetic compounds, 1-heptanol, 3-octanol, linalool oxide, 1-octanol, nonanal, geranyl linalool and phytol, and provide a basis for new inventions on trapping tools for pest management strategies.

Keywords *Momordica cochinchinensis* · Volatiles · Coleoptera · Chrysomelidae · Coccinellidae · Olfactometer bioassay

Introduction

Fruits and leaves of *Momordica cochinchinensis* Spreng (Cucurbitaceae) provide important food products in developing countries. *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae) is an important herbivore pest of *M. cochinchinensis* in Southeast Asian countries such as India, Bangladesh and Vietnam (Singh and Gill 1979; Burke et al. 2005; Lim 2012; Mukherjee et al. 2013, 2014). The insect also feeds on pumpkin, bottle gourd, sponge gourd, etc. (Raman and Annadurai 1985; Rahaman and Prodhan 2007; Khan et al. 2011). Larvae of *A. foveicollis* pass through four instars on young and healthy roots of this plant to complete larval development within 12–13 days. After adults' feeding for 8–9 weeks, the plant finally turns brown (Singh and Gill 1979). Presence of the insect in large numbers results in the death of branches and shoots of this plant, which ultimately reduces crop production. The tachinid fly *Medinodexia morgani*, mite *Histiostoma* sp.

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A. Mukherjee · N. Sarkar · A. Barik (✉)
Ecology Research Laboratory, Department of Zoology, The University of Burdwan, Burdwan 713 104, West Bengal, India
e-mail: anandamaybarik@yahoo.co.in

and reduviid bug *Rhinocoris fuscipes* are recorded as natural enemies of *A. foveicollis* (Crosskey 1973; Waterhouse and Norris 1987), but mass release of the biocontrol agents are not yet successful to control outbreaks of this insect pest. Further, the insect can withstand wide ranges of humidity and temperature, and switches from one crop to another within the same growing season, which causes a serious problem for control of this insect. Farmers are often forced to apply a chemical-based insecticide (Carbofuran, Diazinon–60EC) to control outbreaks of this insect (Sinha and Chakrabarti 1983). *Epilachna dodecastigma* (Wied.) (Coleoptera: Coccinellidae) is another serious pest of bitter gourd and also feeds on *M. cochinchinensis*, pumpkin, bottle gourd, sponge gourd, yardlong bean, potato, etc. in India and Bangladesh (Choudhuri et al. 1983; Hossain et al. 2009; Khan et al. 2011; Sarkar et al. 2013a, 2013b; Sarkar and Barik 2014). The larvae and adults of *E. dodecastigma* start feeding on lower surface of leaves by scrapping, causing net-like appearance of the leaves. Infection by this insect causes death of branches and shoots of this plant (Choudhuri et al. 1983; Hossain et al. 2009). To control pest outbreaks, growers are often forced to use chemical-based insecticides (pyrethroids, organophosphates). To reduce yield losses and environmental risks due to insecticide application, it is a prerequisite to develop new environment friendly products which might be included in integrated pest management (IPM) schemes for this pest.

Plants emit volatile blends which are employed by insect herbivores for host location (Schoonhoven et al. 2005; Bruce and Pickett 2011). The volatile organic compounds (VOCs) in the volatile blends may be ubiquitous in plants, but the specific combination and ratios of VOCs differ between plants species, which results in species-specific attraction (Bruce and Pickett 2011; Magalhães et al. 2012). Further, specificity of VOCs in herbivore-infested plants depends on the type of herbivore and the level of plant infestation (Turlings and Tumlinson 1992; Röse et al. 1996; Paréand Tumlinson 1999; Bruce and Pickett 2011; Magalhães et al. 2012; Sarkar et al. 2014). *Aulacophora foveicollis* females showed attraction toward long-chain fatty acids from *M. cochinchinensis* leaves and flowers (Mukherjee et al. 2014; Mukherjee and Barik 2014), and to long-chain alkanes from flowers of this plant, which are low volatile substances that act as close range attractants after arrival of the insect to the plant (Mukherjee et al. 2013). To date, long-range volatiles from *M. cochinchinensis* leaves, which act as attractants to *A. foveicollis*, have not been identified. Further, herbivore-induced emissions of volatiles play an important role in the olfactorial foraging behaviour of insects (Röse et al. 1996; Schoonhoven et al. 2005). In the present study, volatiles from undamaged plants and mechanically damaged plants, and systematically released feeding-induced volatiles from plants suffering continuous adult conspecific and heterospecific-female feeding were collected by push–pull technique, and subsequently identified and quantified by gas chromatography mass spectrometry (GC-MS) and gas chromatography flame ionization detector (GC-FID) analyses. The behavioural responses of *A. foveicollis* to whole volatile blends from undamaged, conspecific-damaged and heterospecific-damaged *M. cochinchinensis* plants were examined using a Y-shaped glass tube olfactometer bioassay. We further studied the role of individual synthetic volatile components that were characteristic for insect-damaged *M. cochinchinensis* plants as an olfactory cue to *A. foveicollis*. This study indicates that semiochemicals involved in host location may contribute to novel and sustainable pest management programme such as baited traps.

Materials and methods

Insects

Both, *A. foveicollis* and *E. dodecastigma* insects were collected by light trap from *M. cochinchinensis* plants growing in the Crop Research Farm (CRF), The University of Burdwan, and separately maintained in 1-L glass jars, containing bottle gourd (*Lagenaria siceraria* (Molina) Standl.) leaves covered with fine-mesh nylon nets at 27 ± 1 °C temperature, 65 ± 10 % relative humidity and 12 L:12 D photoperiod in a 'BOD' incubator (ADS instruments and Tech., Calcutta, India). To maintain natural condition of leaves, a moist piece of cotton was placed around the cut ends of bottle gourd leaves, wrapped with aluminium foil to prevent moisture loss. Leaves were daily replaced by fresh ones.

Plant materials

Momordica cochinchinensis seeds were germinated on filter paper. Each seed with cotyledon was planted in a pot containing ~150 cm^3 of soil [organic matter 5.3 ± 0.2 % (± Standard Error), pH 7.7, collected from the field of CRF, The University of Burdwan (23°16′N, 87°54′E), West Bengal, India] and held in natural conditions in a climate chamber (photoperiod 13 L:11 D at 30–35 °C) for two months (April–May, 2013). The whole plant with the pot was covered with a clear plastic dome [120 cm (height) × 80 cm (diameter)] to prevent any insect attack and unintentional infection. Plants were provided with water every other day. Two- to three-week-old plants (about 55–60 cm height) with 10 fully expanded leaves were used for volatile collections.

Volatile collections

Momordica cochinchinensis plants were placed into environmental chambers (27 ± 1 °C, 65 ± 5 % RH, and 13 L:11 D) for collection of volatiles from undamaged (UD), mechanically damaged, and *A. foveicollis* and *E. dodecastigma* insect-damaged plants. There were two insect-feeding damage treatments each from *A. foveicollis* or *E. dodecastigma* females: (1) two adult *A. foveicollis* or *E. dodecastigma* females were allowed to feed on only one lower leaf of a *M. cochinchinensis* plant containing ten leaves, and volatiles were collected 24-h post-insect attack from undamaged five upper leaves, and (2) two adult *A. foveicollis* or *E. dodecastigma* females (replaced with a new pair of starved insects every 24 h) were allowed to feed continuously on five lower leaves of a *M. cochinchinensis* plant, and volatiles were collected 120-h post-insect attack from undamaged five upper leaves (Röse et al. 1996). To encourage immediate feeding after being placed to the plants, prior to the feeding assay insects were provisioned with water and starved for 12 h. Volatiles were also collected from five upper leaves of undamaged plants. Plants with undamaged five upper leaves of all treatments were placed individually in 4-L closed glass domes with Teflon bases leaving only a small opening for the stem of the plant. Cotton balls were loosely plugged around the stem of the plant to prevent any abrasion by the Teflon bases. This system helps us to collect volatiles from undamaged five upper leaves, while isolating five lower leaves of the plant where adults were fed in herbivore-damaged treatments. Volatiles from all treatments ($N = 5$ replicates for each treatment) were collected over 10 h during the light phase of photoperiod from 8 AM to 6 PM. Charcoal-filtered air was pushed (6 L min^{-1}) into top of the closed chamber and pulled (1 L min^{-1}) through each volatile collector trap (150 mm long × 5 mm o.d.) containing 80 mg of HayeSep Q (80–100 mesh, Sigma Aldrich, Germany) as an adsorbent, that was inserted into 4 side sampling ports around the base of closed glass chamber.

Volatiles from one single leaf were collected by a specially designed 10 cm diameter × 7 cm height round glass chamber from undamaged, mechanically damaged, and *A. foveicollis* and *E. dodecastigma* insect-damaged plants (Online Resource Fig. 1). Sampling was done using either one leaf or five leaves to observe whether the amounts of volatiles collected were biologically relevant. There were also two insect-feeding damage treatments: (1) two adult *A. foveicollis* or *E. dodecastigma* females were allowed to feed only on the one lower leaf of a *M. cochinchinensis* plant containing ten leaves, and volatiles were collected 24-h post-insect attack from an undamaged upper leaf, and (2) two adult *A. foveicollis* or *E. dodecastigma* females (replaced with a new pair of starved insects every 24 h) were allowed to feed continuously on five lower leaves of a *M. cochinchinensis* plant, and volatiles were collected 120-h post-insect attack from an undamaged upper leaf. Volatiles from all treatments ($N = 5$ replicates for each treatment) were collected over 10 h as mentioned above. Charcoal-filtered air was pushed (1 L min^{-1}) into one side of the round glass chamber and pulled (0.5 L min^{-1}) through volatile collector trap (150 mm long × 5 mm o.d.) containing 80 mg of HayeSep Q as an adsorbent, which is situated 90° apart from the right angle of air entrance (Online Resource Fig. 1).

For the mechanical damage treatments, five lower leaves of *M. cochinchinensis* plants were wounded once with a hole punch, and volatiles were collected right after wounding over 10 h during the light phase of photoperiod between 8 AM and 6 PM from undamaged five upper leaves or one single leaf of *M. cochinchinensis* plants ($N = 5$ replicates for each treatment).

Volatiles were eluted from the adsorbent by washing with 500 µL methylene chloride, and concentrated to 200 µL by a nitrogen flow. One hundred microliters of each extract were used for olfactory bioassays, and the remaining 100 µL was used for chemical analyses. For olfactory bioassays, 20 µL of an aliquot (equivalent to volatiles released by five leaves of a plant in ~1 h) was applied to Whatman No. 41 filter paper (1 cm^2). For quantification through GC, nonyl acetate was added as internal standard (IS), at 20 ng μL^{-1}. All the solvents used were purchased from Sigma Aldrich.

Analysis of volatile samples

Five separate volatile samples from each treatment were analyzed by a Techcomp GC (Em Macau, Rua De Pequim, Nos. 202A-246, Centro Financeiro F7, Hong Kong) model 7900 fitted with an HP-5 capillary column (Agilent; Palo Alto, CA, USA; length: 30 m × 0.25 mm × 0.25 µm film thickness) and a flame ionization detector. The oven temperature programme was initially held at 50 °C for 3 min, then raised at 3.75 °C/min to 240 °C and finally held for 5 min. The carrier gas was nitrogen with a flow rate of 18.5 mL/min. The injector port temperature was 280 °C. One µL sample was injected with a split ratio of 1:10. Components were characterized and quantified against the retention times of authentic standards, which were purchased from Sigma Aldrich.

For further confirmation of identifications, volatiles from each treatment were analyzed with an Agilent 6890 GC coupled to a 5973 Mass Selective Detector with an HP-5 column (same temperature conditions as described for GC analysis). Helium was the carrier gas. One µL sample was injected with a split ratio of 1:10. The MS parameters

were 250 °C at the interface, ionization energy 70 eV, scan speed approximately 1 s. The identity of the volatile compounds was confirmed by comparison of the diagnostic ions and GC retention times with those of respective authentic standards.

Olfactometer bioassays

Aulacophora foveicollis females of different ages were provisioned with water and starved for 10 h prior to use in olfactory bioassays. Age is not considered during olfactory bioassays since the adult females consume leaves of *M. cochinchinensis* plant voraciously for 8–9 weeks until death (Singh and Gill 1979). Females were used in bioassays because they are guided by olfactory cues for both adult feeding and location of suitable larval hosts. The behavioural responses of adult female *A. foveicollis* to *M. cochinchinensis* plant volatiles were investigated in a Y-shaped glass tube olfactometer (15 cm stem and arms long, 0.6 cm radius, 45° Y angle; for modifications see Mukherjee et al. 2014; Mukherjee and Barik 2014). The stem of the olfactometer was connected to a porous glass vial (1 cm radius × 3 cm long) in which test insects were released. Each arm of the olfactometer was connected to a glass-made micro kit adapter fitted into a glass vial (1 cm radius × 3 cm long). One glass vial contained a piece (1 cm^2) of Whatman No. 41 filter paper moistened with 20 µL of volatiles, whilst the other glass vial contained a filter paper of same size moistened with 20 µL of the control solvent (methylene chloride). Charcoal-filtered air was pushed into the system at 300 mL min^{-1}. All the connections between different parts of the setup consisted of silicon tubing.

The effectiveness of volatiles as attractant was evaluated in the following manner in the laboratory at 27 ± 1 °C, 70 ± 3 % relative humidity (RH), and light intensity 150 lux. For each experiment, twenty microliters of volatile sample and the control solvent were applied to separate filter paper pieces, allowed to evaporate and introduced into the glass vials before the first insect was released into olfactometer. One adult female *A. foveicollis* was introduced into the porous glass vial, which was then attached with the stem of the olfactometer and exposed to a particular odour plus one control. The choice behaviour of each female in response to individual synthetic volatile compounds or blend of synthetic volatile compounds was observed for 2 min. This insect was not attracted by the control solvent (methylene chloride) in preliminary assays. A female was considered to have made a choice in case of reaching the end of one arm, the insect was removed from the Y-tube, and the choice of the insect was recorded as a positive or negative response, respectively. In contrast, a female was considered not having made a choice within 2 min, i.e. "non-responding" if it remained in the main arm of the Y-tube until the end of the observation period (Magalhães et al. 2012; Sarkar et al. 2014). Each experiment with one volatile sample was conducted until a total of 90 female insects had responded; and after testing 5 insects the olfactometer setup was cleaned with petroleum ether followed by acetone, and the position of the two arms was systematically changed to avoid positional bias.

Dual choice bioassays with female *A. foveicollis*

Differently treated plant volatile organic compounds (VOCs) tested against solvents controls

Responses of female *A. foveicollis* to volatile organic compounds (VOCs) collected from five upper leaves or one single leaf of differently treated plants (A: undamaged plant, B: plants 24-h post-feeding by *A. foveicollis*, C: plants 120-h post-feeding by *A. foveicollis*, D: plants 24-h post-feeding by *E. dodecastigma*, E: plants 120-h post-feeding by *E. dodecastigma*), respectively, were tested against solvents controls (methylene chloride).

Differently treated plant volatile organic compounds (VOCs) tested against undamaged plants' volatiles

Responses of female *A. foveicollis* to volatile organic compounds (VOCs) collected from five upper leaves or one single leaf of differently damaged plants (A: plants 24-h post-feeding by *A. foveicollis*, B: plants 120-h post-feeding by *A. foveicollis,* C: plants 24-h post-feeding by *E. dodecastigma*, D: plants 120-h post-feeding by *E. dodecastigma*), respectively, were tested against volatiles collected from five upper leaves or one single leaf of undamaged plants.

Differently treated plant volatile organic compounds (VOCs) tested against differently damaged plants' volatiles

Responses of female *A. foveicollis* to volatile organic compounds (VOCs) collected from five upper leaves or one single leaf of conspecifically damaged plants (plants damaged by *A. foveicollis*) were tested against volatiles collected from five upper leaves or one single leaf of heterospecifically damaged plants (plants damaged by *E. dodecastigma*).

Dose-dependent responses to volatile organic compounds (VOCs) of differently treated plants

Responses of female *A. foveicollis* to all volatile components characteristic of post-insect-feeding plants were

assayed against solvents controls, and the compounds that elicited attractions to the insect were also tested at different doses (1-heptanol: 0.50, 1, 2, 4 and 8 μg were separately dissolved in 200 μL methylene chloride, respectively, and 50, 100, 200, 400 and 800 ng/20 μL were used for olfactory bioassays; 3-octanol: 0.375, 0.750, 1.5, 3 and 6 μg were separately dissolved in 200 μL methylene chloride, respectively, and 37.5, 75, 150, 300 and 600 ng/20 μL were used for olfactory bioassays; linalool oxide: 1, 2, 4, 8 and 16 μg were separately dissolved in 200 μL methylene chloride, respectively, and 100, 200, 400, 800 and 1,600 ng/20 μL were used for olfactory bioassays; 1-octanol: 0.2, 0.4, 0.8 and 1.6 μg were separately dissolved in 200 μL methylene chloride, respectively, and 20, 40, 80 and 160 ng/20 μL were used for olfactory bioassays; nonanal: 0.750, 1.5, 3 and 6 μg were separately dissolved in 200 μL methylene chloride, respectively, and 75, 150, 300 and 600 ng/20 μL were used for olfactory bioassays; geranyl linalool: 5, 10, 20 and 40 μg were separately dissolved in 200 μL methylene chloride, respectively, and 500, 1,000, 2,000 and 4,000 ng/20 μL were used for olfactory bioassays; and phytol: 10, 20, 40 and 80 μg were separately dissolved in 200 μL methylene chloride, respectively, and 1,000, 2,000, 4,000 and 8,000 ng/20 μL were used for olfactory bioassays).

Responses to combinations of synthetic compounds (that individually elicited attraction) corresponding to volatile organic compounds (VOCs) of differently treated plants

Finally, the response of the insect to the combination of seven synthetic compounds (Online Resource Table 1a and 1b) in the proportions those are quantified in the five upper leaves or one single leaf of differently treated plants (A: undamaged plant, B: plants 24-h post-feeding by *A. foveicollis*, C: plants 120-h post-feeding by *A. foveicollis*, D: plants 24-h post-feeding by *E. dodecastigma*, E: plants 120-h post-feeding by *E. dodecastigma*), respectively, were tested against solvents controls (methylene chloride).

Chemicals

HayeSep Q (80–100 mesh) was purchased from Sigma Aldrich, Germany. 2-Hexanol, 1-hexanol (≥99.5 %), α-pinene (≥99 %), benzaldehyde (≥99 %), 1-heptanol, 1-octen-3-ol (≥98 %), 3-octanone (≥98 %), 3-octanol (99 %), benzyl alcohol (99.8 %), acetophenone (≥99 %), linalool oxide (≥97 %), 1-octanol (≥99 %), linalool, nonanal, 1-nonanol (≥98 %), decanal, 1-decanol, indol, nerolidol (98 %), 1-hexadecanol, farnesyl acetone (≥90 %), geranyl linalool (≥95 %), and phytol (≥97 %) were purchased from Sigma Aldrich, Germany.

Statistical analyses

The data on total amounts of volatiles and amounts of individual VOCs from five upper leaves or one single leaf of undamaged, mechanically damaged and insect-damaged *M. cochinchinensis* plants were log $(x + 1)$ transformed prior to performing statistical analyses. The log $(x + 1)$ transformed data for total amounts of volatiles and amounts of individual VOCs present in undamaged, mechanically damaged, and 24-h and 120-h post-insect-feeding *M. cochinchinensis* plants were subjected to Levene's test for homogeneity of variance with respect to treatments. In case of homogeneity of variance, one-way ANOVA was conducted to compare the treatment effects on total and individual VOCs. Following this, the data were subjected to post hoc Tukey test using SPSS software (SPSS 16.0; SPSS Inc., Chicago, IL, USA). The data obtained on responses of *A. foveicollis* to VOCs were analyzed by a Chi square test (Roy et al. 2012; Sarkar et al. 2013a, 2013b; Megalhães et al. 2012; Sarkar and Barik 2014). Insects that did not respond to any selection offered in the olfactometer were excluded from the analyses.

Results

Volatiles emitted from five upper leaves of an undamaged, mechanically damaged, and 24- and 120-h post-insect-feeding *M. cochinchinensis* plants (Table 1) as well as from one single leaf of undamaged, mechanically damaged, and 24- and 120-h post-insect feeding plants revealed 22, 21, and 22 compounds, respectively (Table 2). 2-Hexanol was only detected in volatiles of mechanically damaged plants, whereas 1-octen-3-ol and farnesyl acetone were identified in volatiles of undamaged and insect-damaged plants, but not in those of mechanically damaged plants. Total volatile emissions were significantly higher when volatiles were collected from five upper leaves of mechanically damaged plants followed by 120-h post-insect feeding plants, 24-h post-insect feeding plants and undamaged plants ($F = 291.86$; $df = 5, 24$; $P < 0.05$) (Table 3). Similar results were obtained, when volatiles were also collected from one single leaf of *M. cochinchinensis* plants ($F = 364.92$; $df = 5, 24$; $P < 0.05$) (Table 3). Phytol was predominant followed by geranyl linalool in all volatile samples of one upper leaf and five upper leaves (Tables 1 and Table 2). 1-Hexadecanol was least abundant in volatiles of undamaged plants, 24-h post-*A. foveicollis* and *E. dodecastigma* feeding plants, and 120-h post-*E. dodecastigma* feeding plants; whereas 1-hexanol and 2-hexanol were least abundant in volatiles of 120-h post-*A.*

Table 1 GC-FID analysis of VOCs emitted (ng/10 h) by five upper leaves of undamaged, mechanically damaged, and 24-h and 120-h post-insect-feeding *M. cochinchinensis* plants (mean ± SE, $N = 5$)

Peak	Compound	Whole plant	24-h post insect feeding plant		120-h post insect feeding plant		Mechanically damaged plant	$F_{5,24}$	Retention time
			A. foveicollis	*E. dodecastigma*	*A. foveicollis*	*E. dodecastigma*			
1	2-Hexanol	–	–	–	–	–	205.8 ± 12.0		6.95
2	1-Hexanol	206.3 ± 11.9^{a}	256.7 ± 13.0^{b}	242.1 ± 16.1^{ab}	279.8 ± 10.0^{b}	281.2 ± 12.8^{b}	255.9 ± 12.0^{b}	4.76	8.51
3	α-Pinene	222.1 ± 12.8^{a}	270.0 ± 12.2^{bc}	246.4 ± 16.5^{ab}	300.3 ± 10.7 ^{cd}	346.5 ± 23.7^{d}	248.2 ± 11.4^{a}	8.62	8.84
4	Benzaldehyde	232.4 ± 12.6^{a}	752.4 ± 21.3^{b}	470.1 ± 18.9^{c}	956.9 ± 34.9^{d}	495.8 ± 27.8^{c}	246.0 ± 14.1^{a}	167.97	9.61
5	1-Heptanol	3,134.7 ± 80.4	3,192 ± 196.6	3,207.6 ± 201.3	3,226.1 ± 162.9	3,340.9 ± 159.1	3,376.7 ± 242.3	0.24	10.74
6	1-Octen-3-ol	227.1 ± 14.2^{a}	290.0 ± 13.4^{b}	260.5 ± 11.3^{ab}	355.0 ± 15.0^{c}	340.2 ± 11.6^{c}	–	144.76	11.57
7	3-Octanone	3,041.6 ± 72.7	3,095.8 ± 184.8	2,987.8 ± 157.4	3,276.6 ± 152.4	3,435.6 ± 198.6	3,381.3 ± 287.3	0.96	12.11
8	3-Octanol	1,781.9 ± 79.9^{a}	1,789.2 ± 66.4^{a}	2,399.1 ± 138.4^{b}	2,891.4 ± 193.5^{b}	3,052.0 ± 187.7^{b}	1,787.8 ± 120.5^{a}	19.17	12.36
9	Benzyl alcohol	406.7 ± 16.4^{a}	467.7 ± 27.1^{b}	405.5 ± 10.7^{a}	498.6 ± 25.5^{b}	496.8 ± 19.3^{b}	643.5 ± 20.5^{c}	19.13	12.60
10	Acetophenone	777.0 ± 20.4	803.5 ± 28.2	781.0 ± 19.4	825.6 ± 29.0	785.0 ± 47.7	809.8 ± 26.4	0.41	13.68
11	Linalool oxide	4,257.0 ± 104.2^{a}	6,467.1 ± 263.5^{b}	6,786.7 ± 225.6^{bc}	7,076.0 ± 286.3^{bc}	7,703.7 ± 359.0^{c}	7,053.8 ± 367.5^{bc}	25.16	14.33
12	1-Octanol	442.0 ± 16.5^{a}	517.7 ± 20.9^{b}	509.5 ± 14.8^{b}	519.5 ± 15.1^{b}	535.4 ± 25.1^{b}	526.4 ± 19.4^{b}	3.24	15.40
13	Linalool	11,382.2 ± 392.3^{a}	14,164.2 ± 799.8^{bc}	11,230.4 ± 424.2^{a}	15,255.6 ± 602.8^{c}	12,195.9 ± 601.4^{a}	15,174.4 ± 841.6^{c}	9.03	16.24
14	Nonanal	2,581.1 ± 157.3^{a}	2,605.7 ± 112.0^{a}	2,684.3 ± 112.9^{a}	5,310.6 ± 273.0^{b}	5,632.1 ± 280.8^{b}	2,676.3 ± 201.7^{a}	50.43	17.27
15	1-Nonanol	2,445.9 ± 148.1^{a}	3,106.6 ± 111.7^{b}	3,050.0 ± 163.6^{b}	3,565.9 ± 149.2^{b}	3,527.7 ± 252.4^{b}	3,102.0 ± 215.6^{b}	5.52	19.43
16	Decanal	195.5 ± 10.6^{a}	403.3 ± 19.8^{b}	406.9 ± 12.9^{b}	532.1 ± 16.2^{c}	507.2 ± 27.0^{c}	794.0 ± 14.5^{d}	123.72	20.83
17	1-Decanol	1,050.3 ± 49.2^{a}	1,437.5 ± 83.9^{bc}	1,180.7 ± 46.9^{a}	1,571.3 ± 65.8^{c}	1,367.7 ± 47.1^{b}	2,499.9 ± 157.2^{d}	39.08	22.40
18	Indol	411.5 ± 21.6^{a}	428.2 ± 25.2^{ac}	486.2 ± 33.1^{bc}	488.7 ± 26.5^{bc}	493.1 ± 16.8^{bc}	767.7 ± 14.2^{d}	25.75	22.50
19	Nerolidol	487.6 ± 23.2	495.1 ± 21.7	480.5 ± 25.1	500.8 ± 15.5	488.6 ± 19.2	424.2 ± 21.9	1.76	32.48
20	1-Hexadecanol	129.3 ± 6.4^{a}	187.7 ± 12.2^{b}	158.9 ± 9.6^{b}	357.6 ± 11.9^{c}	156.6 ± 7.0^{b}	269.6 ± 18.5^{d}	55.38	38.82
21	Farnesyl acetone	271.9 ± 16.7^{a}	299.1 ± 17.8^{a}	277.8 ± 13.3^{a}	410.1 ± 21.0^{b}	390.0 ± 20.1^{b}	-	102.31	40.84
22	Geranyl linalool	10,998.1 ± 445.0^{a}	15,772.2 ± 646.6^{bc}	14,799.7 ± 665.3^{b}	18,192.4 ± 585.6^{c}	17,799.9 ± 632.7^{c}	9,435.0 ± 627.5^{a}	36.39	43.36
23	Phytol	23,559.8 ± 601.4^{a}	26,226.7 ± 653.0^{a}	25,359.9 ± 697.6^{a}	42,859.2 ± 764.2^{b}	42,397.7 ± 674.2^{b}	152,118.2 ± 3,278.6^{c}	981.79	49.07

Within the rows means followed by different letters are significantly different ($P < 0.05$)

Table 2 GC-FID analysis of VOCs emitted (ng/10 h) by one single leaf of undamaged, mechanically damaged, and 24-h and 120-h post-insect feeding *M. cochinchinensis* plants (mean ± SE, $N = 5$)

Compound	Whole plant	24-h post insect feeding plant		120-h post insect feeding plant		Mechanically damaged plant	$F_{5,24}$
		A. foveicollis	*E. dodecastigma*	*A. foveicollis*	*E. dodecastigma*		
2-Hexanol	–	–	–	–	–	39.7 ± 2.2	
1-Hexanol	40.8 ± 2.6^{a}	49.4 ± 2.8^{b}	45.8 ± 3.0^{ab}	53.0 ± 2.2^{b}	53.5 ± 2.4^{b}	49.0 ± 2.6^{ab}	3.32
α-Pinene	44.2 ± 3.0^{a}	53.6 ± 2.4^{b}	46.5 ± 3.2^{ab}	56.9 ± 2.5^{b}	67.5 ± 4.6^{c}	47.3 ± 2.2^{a}	7.83
Benzaldehyde	46.1 ± 2.7^{a}	144.1 ± 5.8^{b}	89.0 ± 4.0^{c}	183.2 ± 7.6^{d}	93.7 ± 5.2^{c}	47.1 ± 2.5^{a}	123.89
1-Heptanol	618 ± 15.5	604.3 ± 33.1	600.7 ± 34.7	615.3 ± 33.2	631.8 ± 32.3	645.6 ± 44.5	0.25
1-Octen-3ol	44.6 ± 3.0^{a}	57.6 ± 3.4^{a}	49.1 ± 2.4^{a}	68.1 ± 2.6^{b}	64.4 ± 3.7^{b}	–	82.99
3-Octanone	598.7 ± 11.3	586.3 ± 31.0	562.6 ± 28.5	625.4 ± 31.8	649.4 ± 38.1	646.7 ± 53.5	0.99
3-Octanol	351.2 ± 15.6^{a}	338.8 ± 9.9^{a}	451.2 ± 23.3^{b}	549.9 ± 38.6^{c}	576.9 ± 36.0^{c}	342.7 ± 21.8^{a}	17.95
Benzyl alcohol	80.1 ± 3.1^{a}	90.2 ± 6.1^{ab}	76.8 ± 1.7^{a}	94.9 ± 4.8^{b}	76.9 ± 3.6^{a}	123.4 ± 4.0^{c}	18.25
Acetophenone	153.1 ± 3.8	156.1 ± 6.0	147.0 ± 4.4	156.4 ± 7.0	148.9 ± 8.3	155.5 ± 4.6	0.46
Linalool oxide	839.1 ± 19.2^{a}	1,226 ± 47.1^{b}	1,282.6 ± 41.8^{b}	1,344.4 ± 54.5^{bc}	1,457.3 ± 74.8^{c}	1,349.6 ± 66.6^{bc}	19.68
1-Octanol	87.1 ± 3.0^{a}	98.2 ± 3.4^{b}	96.3 ± 2.4^{b}	99.9 ± 4.0^{b}	102.9 ± 6.1^{b}	101.0 ± 3.6^{b}	2.06
Linalool	2,251.5 ± 67.0^{a}	2,682.8 ± 133.0^{b}	2,114.9 ± 77.6^{a}	2,897.4 ± 116.2^{b}	2,305.2 ± 114.5^{a}	2,901.8 ± 152.0^{b}	9.54
Nonanal	510.1 ± 30.0^{a}	493.4 ± 17.9^{a}	509.1 ± 21.4^{a}	1,007.4 ± 47.4^{b}	1,064.1 ± 52.5^{b}	512.8 ± 39.9^{a}	54.73
1-Nonanol	482.2 ± 29.0^{a}	590.2 ± 26.0^{b}	576.9 ± 28.6^{b}	677.2 ± 25.8^{c}	666.7 ± 47.3^{c}	593.4 ± 39.7^{b}	4.51
Decanal	38.6 ± 2.2^{a}	76.5 ± 3.2^{b}	76.6 ± 1.8^{b}	101.1 ± 3.1^{c}	95.9 ± 5.2^{c}	152.0 ± 2.9^{d}	133.47
1-Decanol	203.9 ± 9.9^{a}	272 ± 15.4^{b}	225.3 ± 10.6^{a}	296.3 ± 18.8^{b}	258.3 ± 11.4^{b}	478.3 ± 28.9^{c}	33.06
Indol	81.2 ± 4.3^{a}	81.2 ± 5.1^{a}	95.1 ± 6.8^{a}	93.3 ± 5.6^{a}	92.7 ± 3.3^{a}	147.9 ± 2.0^{b}	26.18
Nerolidol	96.8 ± 4.7	94.1 ± 4.4	90.2 ± 5.2	95.0 ± 2.4	92.4 ± 3.7	81.2 ± 4.0	1.82
1-Hexadecanol	25.7 ± 1.4^{a}	35.6 ± 2.3^{b}	29.9 ± 1.6^{a}	68.1 ± 2.8^{c}	29.6 ± 1.4^{a}	51.6 ± 3.4^{d}	52.13
Farnesyl acetone	53.7 ± 4.2^{a}	63 ± 3.8^{a}	52.5 ± 3.9^{a}	76.9 ± 5.0^{b}	73.1 ± 5.3^{ab}	–	49.30
Geranyl linalool	2,166.1 ± 73.3^{a}	2,991.6 ± 125.2^{b}	2,803.9 ± 126.9^{b}	3,455.0 ± 111.7^{c}	6,727.6 ± 235.9^{c}	1,805.5 ± 115.9^{a}	36.15
Phytol	4,623.0 ± 103.2^{a}	4,962.9 ± 104.6^{a}	4,766.4 ± 152.5^{a}	8,082.3 ± 232.6^{b}	8,012.8 ± 127.8^{b}	29,115.0 ± 542.6^{c}	982.84

Within the rows means followed by different letters are significantly different ($P < 0.05$)

Table 3 Total amounts of volatiles (μg/10 h) (mean ± SE) emitted from five upper leaves and one single leaf of undamaged, mechanically damaged, and 24- and 120-h post-insect-feeding *M. cochinchinensis* plants

Total amount of volatiles	Undamaged	24-h post feeding plant by		120-h post feeding plant by		Mechanically damaged plant	$F_{5,24}$	Sig.
		A. foveicollis	*E. dodecastigma*	*A. foveicollis*	*E. dodecastigma*			
Five upper leaves	68.24 ± 2.31^{a}	83.04 ± 3.34^{b}	78.41 ± 3.04^{b}	109.24 ± 3.47^{c}	105.68 ± 3.65^{c}	205.80 ± 6.52^{d}	291.86	0.0001
Single leaf	13.44 ± 0.41^{a}	15.75 ± 0.59^{b}	14.79 ± 0.59^{b}	20.70 ± 0.76^{c}	19.98 ± 0.71^{c}	39.30 ± 1.14^{d}	364.92	0.0001

Within the rows means followed by different letters are significantly different

foveicollis feeding plants and mechanically damaged plants, respectively (Table 1). Amounts of 1-heptanol, 3-octanone, acetophenone and nerolidol did not differ significantly in the volatiles among undamaged, mechanically damaged and insect-damaged plants. Benzyl alcohol, decanal, 1-decanol, indol, 1-hexadecanol and phytol were released in higher amounts from mechanically damaged plants than the undamaged and insect-damaged plants (Table 1). All other identified VOCs displayed different patterns in undamaged, mechanically damaged and insect-damaged plants (Tables 1 and 2).

Dual choice bioassays with female *A. foveicollis*

Differently treated plant volatile organic compounds (VOCs) tested against solvents controls

Aulacophora foveicollis preferred volatiles from five upper leaves of undamaged plants against controls ($\chi^2 = 16.04$, $df = 1$, $P < 0.0001$), volatiles of plants that had been damaged by conspecifics (*A. foveicollis*), for either 24 h ($\chi^2 = 23.51$, $df = 1$, $P < 0.0001$) or 120 h ($\chi^2 = 30.04$, $df = 1$, $P < 0.0001$) against solvents controls, and plants

that had been damaged by heterospecifics (*E. dodecastigma*), for either 24 h ($\chi^2 = 27.78$, $df = 1$, $P < 0.0001$) or 120 h ($\chi^2 = 42.71$, $df = 1$, $P < 0.0001$) against solvents controls. Moreover, female *A. foveicollis* were attracted to VOCs from one single leaf of *M. cochinchinensis* plants that have been damaged by conspecifics, for either 24 h ($\chi^2 = 5.38$, $df = 1$, $P = 0.02037$) or 120 h ($\chi^2 = 8.71$, $df = 1$, $P = 0.00316$) against solvents controls, and plants that had been damaged by heterospecifics for either 24 h ($\chi^2 = 7.51$, $df = 1$, $P = 0.00613$) or 120 h ($\chi^2 = 12.84$, $df = 1$, $P = 0.00034$) against solvents controls, but did not show a preference for VOCs collected from one single leaf of undamaged plants against solvents controls ($\chi^2 = 0.71$, $df = 1$, $P = 0.39911$).

Differently treated plant volatile organic compounds (VOCs) tested against undamaged plants' volatiles

Female *A. foveicollis* elicited attraction to VOCs collected from five upper leaves of *M. cochinchinensis* plants damaged by conspecifics, at both 24-h ($\chi^2 = 6.4$, $df = 1$, $P = 0.01141$) or 120-h ($\chi^2 = 11.38$, $df = 1$, $P = 0.00074$) against VOCs collected from five upper leaves of undamaged plants, and plants damaged by heterospecifics, at both 24 h ($\chi^2 = 8.71$, $df = 1$, $P = 0.00316$) or 120 h ($\chi^2 = 16.04$, $df = 1$, $P < 0.0001$) against VOCs collected from five upper leaves of undamaged plants; whereas females showed preference to VOCs collected from one single leaf of *M. cochinchinensis* plants damaged by conspecifics, at both 24 h ($\chi^2 = 4.44$, $df = 1$, $P = 0.03502$) or 120 h ($\chi^2 = 6.4$, $df = 1$, $P = 0.01141$) against VOCs from one single leaf of an undamaged plant, and plants damaged by heterospecifics, at both 24 h ($\chi^2 = 5.38$, $df = 1$, $P = 0.02037$) or 120 h ($\chi^2 = 8.71$, $df = 1$, $P = 0.00316$) against VOCs from one single leaf of an undamaged plant. The results indicated that volatiles from five upper leaves or one single leaf of insect-damaged plants caused higher attraction of *A. foveicollis* than volatiles from five upper leaves or one single leaf of undamaged plants.

Differently treated plant volatile organic compounds (VOCs) tested against differently damaged plants' volatiles

Female *A. foveicollis* were more attracted to VOCs from five upper leaves of *M. cochinchinensis* plants that have been damaged by heterospecifics for 120 h ($\chi^2 = 7.51$, $df = 1$, $P = 0.00613$) than to volatiles of conspecifically damaged, whereas females showed no preference for VOCs to 24 h heterospecific-damaged volatiles against 24 h conspecific-damaged volatiles ($\chi^2 = 3.6$, $df = 1$, $P = 0.05778$). Females showed no preference for VOCs collected from one single leaf of *M. cochinchinensis* plants damaged by heterospecifics at both 24 h ($\chi^2 = 0.04$, $df = 1$, $P = 0.83385$) or 120 h ($\chi^2 = 1.11$, $df = 1$, $P = 0.29186$) against conspecific-damaged volatiles.

Dose-dependent responses to volatile organic compounds (VOCs) of differently treated plants

In Y-tube olfactory bioassays, 7 individual compounds, 1-heptanol, 3-octanol, linalool oxide, 1-octanol, nonanal, geranyl linalool and phytol that were found to be characteristic for volatiles of undamaged and insect-damaged *M. cochinchinensis* plants elicited attraction of the test insect (versus solvents controls) (Table 4), whereas rest of the identified 15 compounds in the volatiles of 120-h post-insect-feeding plants showed no preference to the insect. 1-Heptanol was attractive in concentrations of 100 ng/20 µL CH_2Cl_2, 200, 400 and 800 ng/20 µL (Table 4). Application of 3-octanol resulted attraction in concentrations of 75 ng/20 µL CH_2Cl_2, 150, 300 and 600 ng/20 µL (Table 4). Female *A. foveicollis* was attracted to linalool oxide in concentrations of 200 ng/20 µL CH_2Cl_2, 400, 800 and 1,600 ng/20 µL (Table 4). 1-Octanol showed attraction in concentrations of 40 ng/20 µL CH_2Cl_2, 80 and 160 ng/20 µL (Table 4). Nonanal elicited attraction in concentrations of 150 ng/20 µL CH_2Cl_2, 300 and 600 ng/20 µL (Table 4). Geranyl linalool was preferred in concentrations of 1,000 ng/20 µL CH_2Cl_2, 2,000 and 4,000 ng/20 µL (Table 4). Phytol was attractive in concentrations of 2,000 ng/20 µL CH_2Cl_2, 4,000 and 8,000 ng/20 µL (Table 4).

Responses to combinations of synthetic compounds (that individually elicited attraction) corresponding to volatile organic compounds (VOCs) of differently treated plants

Aulacophora foveicollis were attracted to a synthetic blend of seven volatile components equivalent to seven volatile components of five upper leaves of undamaged plants against solvents controls, blends of seven synthetic volatile components equivalent to the proportions in plants that had been damaged by conspecifics (*A. foveicollis*), for either 24 or 120 h against solvents controls, and plants that had been damaged by heterospecifics (*E. dodecastigma*), for either 24 or 120 h against solvents controls (Fig. 1a). Moreover, female *A. foveicollis* were attracted to blends of seven synthetic volatile components equivalent to the proportions of one single leaf of *M. cochinchinensis* plants that have been damaged by conspecifics or heterospecifics for 120 h against solvents controls, but did not show a preference for a synthetic blend of seven volatile components equivalent to the proportions of one single leaf of undamaged plants against solvents controls, and plants that have been damaged by conspecifics or heterospecifics for 24 h against solvents controls (Fig. 1b). The results

Table 4 Female *A. foveicollis* responses to individual synthetic volatile component vs. solvents (CH_2Cl_2) controls in Y-tube olfactometer bioassay ($N = 90$ in each concentration bioassay)

Synthetic compounds	Concentration (ng/20 μL)	χ^2 ($df = 1$)	P values of insect responded
1-Heptanol	50	0.04	0.83385
	100	4.44	0.03502
	200	8.71	0.00316
	400	12.84	0.00034
	800	19.6	<0.0001
3-Octanol	37.5	1.11	0.29186
	75	4.44	0.03502
	150	8.71	0.00316
	300	14.4	0.00015
	600	17.78	<0.0001
Linalool oxide	100	0.71	0.39908
	200	4.44	0.03502
	400	7.51	0.00613
	800	12.84	0.00034
	1,600	19.6	<0.0001
1-Octanol	20	1.11	0.29186
	40	5.38	0.02037
	80	7.51	0.00613
	160	16.04	<0.0001
Nonanal	75	1.6	0.20590
	150	6.4	0.01141
	300	11.38	0.00074
	600	16.04	<0.0001
Geranyl linalool	500	0.71	0.39908
	1,000	4.44	0.03502
	2,000	11.38	0.00074
	4,000	17.78	<0.0001
Phytol	1,000	1.6	0.20590
	2,000	5.38	0.02037
	4,000	8.71	0.00316
	8,000	16.04	<0.0001

revealed that synthetic blends of seven compounds (1-heptanol, 3-octanol, linalool oxide, 1-octanol, nonanal, geranyl linalool and phytol) equivalent to the proportions of one single leaf of plants that have been damaged for 24 h by either conspecifics or heterospecifics synergistically failed to produce similar response pattern like that of volatiles released from five upper leaves of 24-h insect-damaged plants.

Discussion

The present study demonstrates a total of 22, 21 and 22 VOCs in blends of undamaged, mechanically damaged and insect-damaged *M. cochinchinensis* plant leaves, respectively. This study indicates higher amounts of phytol followed by geranyl linalool, linalool, linalool oxide and many other compounds that were also identified in different parts of *M. charantia* plants (Fernando and Grün 2001; Moronkola et al. 2009; Sarkar et al. 2014). In a similar study, the major VOCs emitted by fruits and vines of *M. charantia* have been found to contain myrtenol, (*Z*)-3-hexenol, benzyl alcohol, 1-penten-3-ol, (*Z*)-2-pentenol, (*E*)-2-hexenal and *cis*-sabinol, and the volatiles elicited attraction of the fly *Dacus cucurbitae* (Binder et al. 1989). Further, 1-tridecanol was detected in higher amounts followed by phytol in *M. charantia* leaf volatiles, and individual geraniol, 1-tridecanol and phytol indicated attraction of *E. dodecastigma* (Sarkar et al. 2014).

Our study reveals that herbivore feeding results in an increase in the total emission of volatile compounds, with variable strengths of responses referring to different types of feeding damage (Paré and Tumlinson 1996; Röse et al. 1996; Piesik et al. 2011; Magalhães et al. 2012; Piesik et al. 2013). The olfactometric bioassay results clearly indicate that the test insect, *A. foveicollis* could discriminate between the whole volatile blends released from either conspecific- or heterospecific-damaged *M. cochinchinensis* plants and those released from undamaged *M. cochinchinensis* plants. *Aulacophora foveicollis* are strongly attracted by volatiles of insect-damaged plants, irregardless of the number of leaves damaged. Only whole volatile blends from one single leaf of undamaged *M. cochinchinensis* plants were not attractive to the insect. Preferentially *A. foveicollis* respond to heterospecific-damaged *M. cochinchinensis* plants. This implicates that infestation by *E. dodecastigma* in *M. cochinchinensis* plants in the field might cause further attraction of *A. foveicollis* in the crop field. This may be due to defensive actions of the plant are reduced as a result of *E. dodecastigma* attack, and this supersedes the possible negative effects of competition with *E. dodecastigma* (Pallini et al. 1997; Dugravot et al. 2007). However, *A. foveicollis* attack in *M. cochinchinensis* plants might also lead to further attraction of the insect in the field, which ultimately would reduce crop production. In conclusion, this study supports previous observations that coleopterans prefer to attract previously infested plants (Bolter et al. 1997; Landolt et al. 1999). Attraction of *A. foveicollis* to conspecific-damaged *M. cochinchinensis* plants may refer to increased emissions of several compounds that were also present in whole volatile blends of undamaged plants, including benzyl alcohol and linalool. It is widely known that benzyl alcohol and linalool might play an important role in plant defense induced by herbivory such as facilitating attraction of natural enemies to the insect pest (De Moraes et al. 1998; Tabata et al. 2011). In the present study, higher amounts of

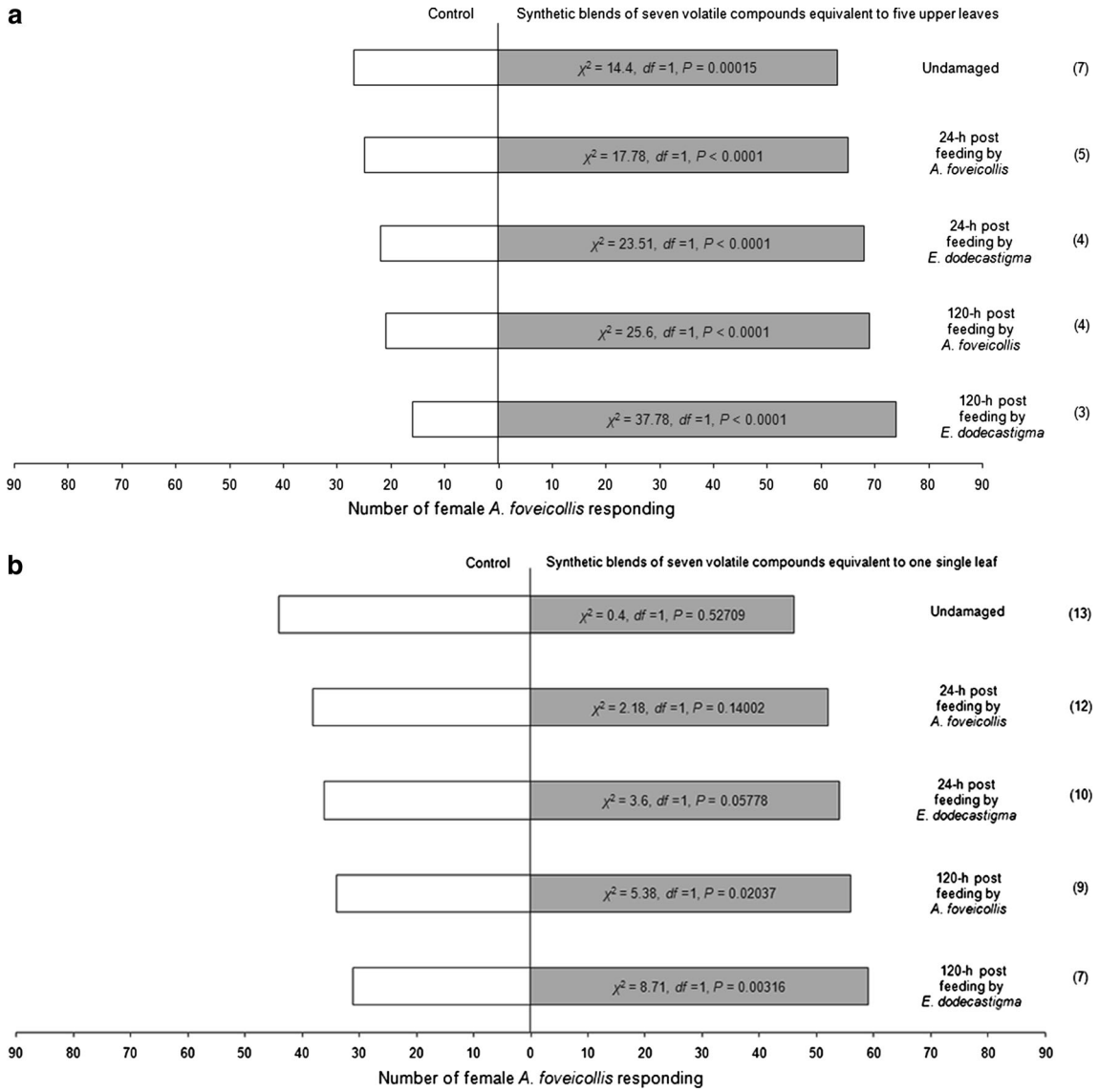

Fig. 1 Female *A. foveicollis* responses to a blend of seven synthetic volatile components (1-heptanol, 3-octanol, linalool oxide, 1-octanol, nonanal, geranyl linalool and phytol) equivalent to the proportions of **a** five upper leaves of undamaged plants or plants 24-h post-insect feeding or plants 120-h post-insect feeding vs. solvents (CH_2Cl_2) controls, **b** one single leaf of undamaged plants or plants 24-h post-insect feeding or plants 120-h post-insect feeding vs. solvents (CH_2Cl_2) controls in Y-tube olfactometer bioassay. *Numbers in brackets* are the number of insects that did not respond to either treatment

these compounds were released by plants 120-h post-*A. foveicollis* feeding than by undamaged and 24-h post-insect-feeding plants (Table 3). But, *E. dodecastigma* attack on *M. cochinchinensis* plants did not reveal any differences in the concentrations of linalool between undamaged and *E. dodecastigma* feeding plants. However, the present olfactory bioassay results demonstrate no significant attraction of *A. foveicollis* to synthetic benzyl alcohol and linalool when these compounds were tested against solvents controls. Insects employ compounds between the ranges of 3 and10 as host location cue (Bruce and Pickett 2011). This study demonstrated that *A. foveicollis* females could detect seven compounds, 1-heptanol, 3-octanol, linalool oxide, 1-octanol, nonanal, geranyl linalool and phytol at the minimal concentrations of 100 ng/20 µL CH_2Cl_2, 75, 200, 40, 150, 1,000 and 2,000 ng/20 µL, respectively. In the ecological context, the small amounts of volatiles might be ubiquitous compounds as it might be produced by other plants in the habitat (Bruce et al. 2005; Bruce and Pickett 2011). Hence, the ratio of volatiles released by the *M. cochinchinensis* plants becomes vital components which act as olfactory cue for *A. foveicollis* (Bruce et al. 2005; Bruce and Pickett 2011). However, the attraction to the overall blend of plant-

derived VOCs released by insect-damaged plants cannot be ruled out, as insect responses to olfactory foraging cues depend on overall volatile blend rather than on attraction of individual compounds (Riffell et al. 2009; Webster et al. 2010). Visual cues from *M. cochinchinensis* plants might also play a role in the attraction, but these cues were not present in the olfactometer bioassay.

An understanding of the signals that act as cues for host plant location by adults might be used for the development of pest management strategies such as baited traps. These findings document that 100 ng/20 μL CH_2Cl_2, 75, 200, 40, 150, 1,000 and 2,000 ng/20 μL of 1-heptanol, 3-octanol, linalool oxide, 1-octanol, nonanal, geranyl linalool and phytol, respectively, might facilitate in the development of much needed eco-friendly trapping tools for pest management of *A. foveicollis*.

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