

Electroantennographic and Behavioral Responses of *Bactrocera cucurbitae* (Coquillett) to Volatile Compounds of *Luffa acutangula* L

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Abstract

The melon fly, *Bactrocera cucurbitae* (Coquillett), is an invasive pest of fruits and vegetables and. Melon fly attractants have been used in control strategies to reduce pest levels and have gained widespread attention in recent years. Previous studies have shown that some host fruits are attractive to melon flies; however, few have investigated the chemical compounds responsible for their attraction. In this study, volatile compounds from *Luffa acutangula* L. were isolated and identified using dynamic headspace adsorption and gas chromatography-mass spectrometry (GC-MS), and their effects on *B. cucurbitae* were investigated using electroantennography (EAG) and behavioral tests (trap assays). Chemical profiling identified 21 compounds in ridge gourds, with stearyl alcohol, methyl myristate, phenyl acetate, and methyl stearate being the major components. The EAG results revealed that 12 compounds elicited antennal responses in *B. cucurbitae*, and the differences in antennal responses between male and female *B. cucurbitae* adults caused by heptanal, phenyl acetate, p-xylene, alpha-pinene, and 1-octadecene were significant. Moreover, in the behavioral experiment, the EAG-active compounds methyl isovalerate, methyl myristate, 1-octadecene, methyl laurate, heptanal, and 1-decanol had either attractive or repellent effects on *B. cucurbitae*. Our findings provide a theoretical basis producing repellents or attractants for effective green control of *B. cucurbitae*.

1. Introduction

The melon fly *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) is native to India and widely distributed in tropical and subtropical regions worldwide (Dhillon et al., 2005; Mir et al., 2014). Infestations are particularly serious in cucurbit crops, with fruit losses ranging from 30–100% (Christenson et al., 1960; Dhillon et al., 2005). Currently, melon fly infestations are primarily controlled using insecticides; however, this is not a suitable control method owing to high levels of insecticide resistance and pesticide residues, which have harmful effects on human health and the environment (Curl et al., 2020; Cha et al., 2023; Hsu et al., 2021; Aktar et al., 2009). Moreover, the control of melon flies is mainly aimed at male insects. Although several male attractants are known, such as raspberry ketone and melolure, populations can recover easily (Lehman et al., 2019; Stringer et al., 2019; Jang et al., 2017). Females lay eggs on the surface of fruits, which is the main cause of harm, and the chemicals that attract females are food-bait attractants, such as fermented sugars, hydrolyzed proteins, and yeast, with limited success (Vargas et al., 2015; Barry et al., 2006; Vargas et al., 2018; Dhillon et al., 2005). Exploring the use of host plant kairomones to attract melon flies is a promising choice for an integrated management plan of control.

Most plants have volatile chemical signals that affect insect behavior, such as locating hosts, oviposition sites, mates, and avoiding predators (Leal et al., 2013; Hansson et al., 2011; Suh et al., 2014; Lin et al., 2015; Wang et al., 2016), which can play important roles in plant-insect interactions (Germain et al., 2010; Nascimento et al., 2021; Song et al., 2020). Host plant volatiles are known to be used as cues by female *B. cucurbitae* to locate potential hosts and have been studied as attractive sources for detection and control (Piñero et al., 2020; Piñero et al., 2021; Vargas et al., 2018). Previous studies have shown that several host

fruits attract gravid female melon flies (Subhash et al.,2018; Piñero et al., 2021; Siderhurst et al.,2010; Jang et al., 2017). The behavioral response of insect species to olfactory cues is not only affected by environmental conditions but also by age, life history, and other physiological states (Epsky et al.,2014; Piñero et al., 2021). Melon flies seek food sources during the pre-oviposition period and host fruits after mating. Previous studies have reported that ridge gourd volatiles are attractive to gravid female melon flies, however, their effect before mating remains unclear (Shivaramu et al.,2022).

Therefore, we investigated the attraction of immature male and female adult *B. cucurbitae* to volatiles released from ridge gourds. Specifically, we used electrophysiological, chemical, and behavioral responses to identify the chemicals that mediate the interaction between melon flies and ridge gourds. The results of this study provide a basis for developing new strategies for using attractants for field trapping.

2. Materials and Methods

2.1. Insects

Melon flies were obtained from rotten fruits in the fields around Guangxi and kept in insect rearing cages (30 cm × 30 cm × 30 cm) at ambient lab conditions ($26 \pm 1^\circ\text{C}$, $70 \pm 10\%$ relative humidity and 14:10 light: dark cycle) for adults to emerge and be maintained for over 20 generations. The emerged adults were fed water and forage (yeast: sugar = 1:2), which were renewed every five days. Three-to seven-day-old unmated female and male adults were used for the electroantennography (EAG) and behavioral experiments. Before the EAG and behavioral experiments, the insects were starved for 6 h and kept in the absence of food odors.

2.2. Host plants

The ridge gourd (provided by the Institute of Vegetables, Guangxi Academy of Agricultural Sciences) was planted in the Beafu South Crop Integrated Cultivation Base of Nanning City, Guangxi Zhuang Autonomous Region, under conventional water and fertilizer management. Fruits with consistent ripeness and no damage were used for headspace volatile collections.

2.3. Odor stimuli

Each chemical of volatiles was dissolved in n-hexane to prepare 0.01, 0.1, 1, 10, and 100 $\mu\text{g}/\mu\text{L}$ solutions, and the solutions were stored at -20°C until required; n-hexane was used as a negative control. All chemicals used in this study were purchased from Sigma-Aldrich (USA).

2.4. Collection of plant volatiles

Dynamic headspace sampling (DHS) was used to collect volatiles from ridge gourds. Before use, the sampling cylinder, gas-washing cylinder, silicone tube, and other instruments were cleaned with 75% absolute ethanol and heated at 180°C for 6 h to eliminate odors. For volatile collection, an air stream was pulled from the top and bottom ports of the jar. The vacuum line was connected to two glass collection

cartridges, each containing 200 mg of the PoraPak Q polymer adsorbent, and charcoal-filtered air was pumped into the tank at a rate of 4 L/min. For each growth stage, volatiles were sampled four times; air volatiles without plants were collected under the same conditions as the blank control, and volatiles were collected for 24 h. Samples were eluted from the adsorbent immediately with 2 mL of n-hexane and concentrated to 0.5 mL. The extracted samples were stored at -20°C until use.

2.5. Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) (Agilent, 7890N-5975C) was performed on a DB-5MS column (30 m × 250 µm × 0.25 µm, Agilent), and analyses of the extracts were carried out under splitless conditions: the injecting sample was 1 µL, carrier gas was helium, and the rate of gas flow was 1 L/min. The column temperature was programmed as follows: 40°C for 5 min, 10°C/min to 150°C for 3 min, and 5°C/min to 250°C for 5 min. Ionization was achieved via electron impact at 70 eV and 250°C. The detected compounds in the volatiles were preliminarily identified by comparison with mass spectra in the NIST17.L library database, retention index (Grajek et al., 1999), and retention times of standard compounds.

2.6. Electroantennography (EAG)

A dose-response relationship was detected using EAG (Syntech, The Netherlands) for individual volatiles. The head of *B. cucurbitae* was excised with a blade, and the tip of the antenna (1 mm) was cut off. The antenna was installed between two glass capillary electrodes filled with 0.1 mol/L KCl. Each test solution (10 µL) was applied to filter paper strips (40 mm breadth × 5 cm length) that served as the olfactory stimuli. The filter paper was placed into a 10-cm sample tube with the end connected to the odor stimulus control device. Air (400 mL/min) filtered by charcoal was constantly directed onto the antennal portions through a stainless-steel delivery tube, and the outlet was approximately 1 cm away from the antenna. Using a stimulus controller to provide 0.5 s air-pulse, an antenna was stimulated for 0.5 s, and the interval between each stimulation was 30 s, which guaranteed the recovery of antennae receptors. The order of stimulation was n-hexane, then the tested volatiles, n-hexane and EAG responses to n-hexane before and after the test chemicals were used as controls. The EAG responses to these two controls were averaged, and the mean was subtracted from the responses to the corresponding dilution series to correct for the solvent. For each compound, five concentrations (from 0.01 to 100 µg/µL) were tested on at least fifteen different females and males, and each antenna was tested only once.

2.7. Behavioral Bioassays

Trap assays were performed as previously described (Qiao et al., 2019). The melon fly (100 females and 100 males, 3–7 d old, starved for 6 h) were introduced into a test chamber, comprising a rearing cage (30 cm × 30 cm × 30 cm), and two 150 mL white transparent plastic cups (6.5 cm × 6.5 cm × 4 cm) with a cut pipette tip (tip opening, 0.5 cm) were placed in two diagonally opposing corners. One of the cups was absorbent cotton containing 1 mL n-hexane (control), whilst the other cup contained 1 mL of the odorants (prepared at five doses 0.01, 0.1, 1, 10, and 100 µg/µL). The numbers of *B. cucurbitae* inside and outside the traps were counted after 24 h. The odorant trap was recorded as an attractive choice, whilst the n-

hexane trap was recorded as repellent. Each experiment was repeated at least five times. Experiments began at the same time each day and were carried out in a climate chamber programmed for a 14L:10D photoperiod, constant temperature of $26 \pm 1^\circ\text{C}$, and $70 \pm 10\%$ relative humidity.

2.8. Data analysis

All statistical analyses were performed using IBM SPSS20. The EAG responses were analyzed using one-way ANOVA with Tukey's multiple comparison tests. Trap responses were analyzed by χ^2 (Chi-square) test. Differences were considered significant at $P < 0.05$. All illustrations were performed using Prism 8.

3. Results

3.1. Chemical analysis of *L. acutangula* volatiles

The GC-MS results revealed that *L. acutangula* has 21 volatile components, including alkenes, esters, aldehydes, alcohols, alkanes, and aromatics. Methyl palmitate, 1-octadecene, and methyl stearate were the major components with contents of 15.4%, 14.1%, 6.08%, and 4.66%, respectively (Table 1).

Table 1
Identification of volatile compounds of *L. acutangular*

Number ^a	RI ^b	Retention times ^c	Name	Abundance (%)	CAS
1	862	7.47	p-xylene	0.49	106-42-3
2	931	10.07	alpha-pinene	0.28	80-56-8
3	1302	30.88	tridecene	1.21	2437-56-1
4	1500	31.38	pentadecane	0.94	629-62-9
5	1196	33.10	β -cyclocitral	0.97	432-25-7
6	1600	33.29	hexadecane	3.12	544-76-3
7	2052	33.74	linoleyl alcohol	1.63	506-43-4
8	1882	34.52	1-hexadecanol	2.13	36653-82-4
9	882	34.54	heptanal	4.36	111-71-7
10	1790	34.70	1-octadecene	3.97	112-88-9
11	1707	35.17	methyl myristate	5.21	124-10-7
12	1816	36.20	phytane	1.69	638-36-8
13	2070	37.04	stearyl alcohol	21.40	112-92-5
14	1918	37.53	methyl palmitate	19.02	112-39-0
15	770	39.53	methyl isovalerate	5.69	556-24-1
16	2117	39.87	methyl stearate	14.76	112-61-8
17	1033	42.54	phenyl acetate	6.18	122-79-2
18	1509	42.78	methyl laurate	2.19	111-82-0
19	1208	49.08	citronellol	1.12	106-22-9
20	1256	49.53	1-decanol	0.93	112-30-1
21	1006	51.57	benzyl alcohol	0.79	100-51-6

^aNumber of a compound. ^bRetention indices calculated according to Kováts. ^cRetention time.

3.2. EAG responses

Of all the 21 compounds, twelve of the EAG-active compounds, namely, heptanal, phenyl acetate, methyl stearate, 1-decanol, methyl myristate, citronellol, p-xylene, phytane, α -pinene, methyl isovalerate, 1-octadecene and methyl laurate, were detected, as shown in Fig. 1. Among them, heptanal elicited high EAG responses and methyl laurate elicited weak EAG responses of *B. cucurbitae* adult males and

females. In addition, the EAG responses induced by heptanal, phenyl acetate, p-xylene, α -pinene, and 1-octadecene were significantly different between adult females and males (heptanal: $F = 13.876$, $df = 11$, $P < 0.001$; phenyl acetate: $F = 12.647$, $df = 11$, $P < 0.001$; methyl stearate: $F = 3.22$, $df = 11$, $P = 0.075$; 1-decanol: $F = 4.453$, $df = 11$, $P = 0.053$; methyl myristate: $F = 3.168$, $df = 11$, $P = 0.067$; p-xylene: $F = 16.857$, $df = 11$, $P < 0.001$; phytane: $F = 4.746$, $df = 11$, $P = 0.051$; methyl isovalerate: $F = 4.364$, $df = 11$, $P = 0.056$; 1-octadecene: $F = 7.109$, $df = 11$, $P = 0.009$; methyl laurate: $F = 2.394$, $df = 11$, $P = 0.136$).

The dose responses of EAG-active volatiles are shown in Fig. 2. Generally, the antennal responses of both sexes increased as the volatile concentrations increased from 0.01 to 100 $\mu\text{g}/\mu\text{L}$. However, there were different threshold values for the antennal responses of *B. cucurbitae* to different volatiles. The antennal responses of *B. cucurbitae* to heptanal, phenyl acetate, methyl stearate, p-xylene, phytane, and citronellol reached its maximum at 100 $\mu\text{g}/\mu\text{L}$ (Fig. 2a-f). The antennal responses of *B. cucurbitae* to methyl myristate, α -pinene, methyl isovalerate, and 1-octadecene reached a maximum at 10 $\mu\text{g}/\mu\text{L}$ (Fig. 2g-j). The antennal responses of *B. cucurbitae* to 1-decanol and methyl laurate reached a maximum at 1 $\mu\text{g}/\mu\text{L}$ (Fig. 2k-l) (heptanal: $F = 55.649$, $df = 4$, $P < 0.001$, male $F = 49.702$, $df = 4$, $P < 0.001$; phenyl acetate: female $F = 44.978$, $df = 4$, $P < 0.001$, male $F = 32.921$, $df = 4$, $P < 0.001$; methyl stearate: female $F = 30.298$, $df = 4$, $P < 0.001$, male $F = 30.664$, $df = 4$, $P < 0.001$; p-xylene: female $F = 41.603$, $df = 4$, $P < 0.001$, male $F = 48.721$, $df = 4$, $P < 0.001$; phytane: female $F = 16.629$, $df = 4$, $P < 0.001$, male $F = 10.887$, $df = 4$, $P < 0.001$; citronellol: female $F = 19.449$, $df = 4$, $P < 0.001$, male $F = 14.442$, $df = 4$, $P < 0.001$; methyl myristate: female $F = 14.171$, $df = 4$, $P < 0.001$, male $F = 10.606$, $df = 4$, $P < 0.001$; α -pinene: female $F = 12.142$, $df = 4$, $P = 0.009$, male $F = 3.361$, $df = 4$, $P = 0.016$; methyl isovalerate: female $F = 7.902$, $df = 4$, $P < 0.001$, male $F = 3.507$, $df = 4$, $P = 0.013$; 1-octadecane: female $F = 14.473$, $df = 4$, $P < 0.001$, male $F = 11.073$, $df = 4$, $P < 0.001$; 1-decanol: female $F = 11.006$, $df = 4$, $P < 0.001$, male $F = 10.312$, $df = 4$, $P < 0.001$; methyl laurate: female $F = 13.970$, $df = 4$, $P = 0.013$, male $F = 11.221$, $df = 4$, $P < 0.001$).

3.3. Behavioral responses

In the behavioral trapping experiment, melon flies of both sexes showed significant sex- and concentration-dependent responses to the twelve EAG-active compounds tested separately. At all test concentrations, methyl isovalerate was attractive to both sexes of *B. cucurbitae* at high concentrations: 100 $\mu\text{g}/\mu\text{L}$ (methyl isovalerate: female $\chi^2 = 43.781$, $df = 1$, $P < 0.001$, male $\chi^2 = 12.645$, $df = 1$, $P < 0.001$), 10 $\mu\text{g}/\mu\text{L}$ (female $\chi^2 = 12.980$, $df = 1$, $P < 0.001$, male $\chi^2 = 20.275$, $df = 1$, $P < 0.001$), and 1 $\mu\text{g}/\mu\text{L}$ (female $\chi^2 = 23.629$, $df = 1$, $P < 0.001$, male $\chi^2 = 27.125$, $df = 1$, $P < 0.001$) (Fig. 3–5). However, it was repellent to both sexes at low concentrations: 0.1 and 0.01 $\mu\text{g}/\mu\text{L}$. 0.1 $\mu\text{g}/\mu\text{L}$ (female $\chi^2 = 26.611$, $df = 1$, $P < 0.001$, male $\chi^2 = 17.887$, $df = 1$, $P < 0.001$), and 0.01 $\mu\text{g}/\mu\text{L}$ (female $\chi^2 = 19.444$, $df = 1$, $P < 0.001$, male $\chi^2 = 25.293$, $df = 1$, $P < 0.001$) (Fig. 6–7). In addition, sex differences were apparent in the response of *B. cucurbitae* to methyl myristate, which changed from having a repellent (10 $\mu\text{g}/\mu\text{L}$) to an attractive effect (1–0.01 $\mu\text{g}/\mu\text{L}$) for females and from attractive (10 $\mu\text{g}/\mu\text{L}$) to repellent (1 $\mu\text{g}/\mu\text{L}$) for males (10 $\mu\text{g}/\mu\text{L}$: female $\chi^2 = 16.629$, $df = 1$, $P < 0.001$, male $\chi^2 = 12.500$, $df = 1$, $P < 0.001$; 1 $\mu\text{g}/\mu\text{L}$: female $\chi^2 = 15.672$, $df = 1$, $P < 0.001$, male $\chi^2 = 13.530$, $df = 1$, $P < 0.001$; 0.1 $\mu\text{g}/\mu\text{L}$: female $\chi^2 = 13.235$, $df = 1$, $P < 0.001$; 0.01 $\mu\text{g}/\mu\text{L}$: female $\chi^2 = 13.235$,

$df = 1$, $P < 0.001$) (Fig. 4–7). Additionally, heptanal was attractive to both sexes of *B. cucurbitae* at 100 $\mu\text{g}/\mu\text{L}$ (female $\chi^2 = 13.026$, $df = 1$, $P < 0.001$, male $\chi^2 = 22.733$, $df = 1$, $P < 0.001$) (Fig. 3) and repellent to males at 10 $\mu\text{g}/\mu\text{L}$ (male $\chi^2 = 17.921$, $df = 1$, $P < 0.001$) (Fig. 4); 1-decanol was attractive to females at 1 $\mu\text{g}/\mu\text{L}$ (female $\chi^2 = 6.666$, $df = 1$, $P = 0.01$) (Fig. 5) and 0.1 $\mu\text{g}/\mu\text{L}$ (female $\chi^2 = 9.758$, $df = 1$, $P = 0.002$) (Fig. 6). Furthermore, 1-octadecene changed from having an attractive effect (0.1 $\mu\text{g}/\mu\text{L}$) to a repellent one (0.01 $\mu\text{g}/\mu\text{L}$) on females (0.1 $\mu\text{g}/\mu\text{L}$: $\chi^2 = 7.219$, $df = 1$, $P = 0.007$; 0.01 $\mu\text{g}/\mu\text{L}$: $\chi^2 = 21.597$, $df = 1$, $P < 0.001$) (Fig. 6–7), and methyl laurate was attractive to both sexes of *B. cucurbitae* at low concentrations: 0.1 $\mu\text{g}/\mu\text{L}$ (female $\chi^2 = 12.578$, $df = 1$, $P < 0.001$, male $\chi^2 = 14.713$, $df = 1$, $P < 0.001$), and 0.01 $\mu\text{g}/\mu\text{L}$ (female $\chi^2 = 21.962$, $df = 1$, $P < 0.001$, male $\chi^2 = 26.264$, $df = 1$, $P < 0.001$) (Fig. 6–7).

4. Discussion

Volatile chemical signals released by host plants are important for melon flies to find suitable hosts, feed, and oviposit. Melon flies prefer cucurbits as host plants, and their attraction to several fruits, including cucumbers, bitter gourds, snake gourds, and zucchini, has been documented (Subhashet al., 2018; Piñero et al., 2019). However, only cucumber volatiles have been studied as attractants of female *B. cucurbitae* (Siderhurst and Jang 2010). Therefore, the search for attractants from other hosts is important.

Thus far, compounds emitted by ridge gourd have been studied fragmentedly, six and EAG-active compounds were identified as α -pinene, 1-octen-3-ol, pcymene, p-ethyl-benzaldehyde, methyl salicylate and p-cymen-7-ol (Shivaramu et al., 2022). In the current study, twelve types of EAG-active compounds were identified, including α -pinene. The low similarity with our results could be due to both methodological differences and a significant difference in temperature systems used for the collection of compounds, ridge gourd varieties, absorbents, and plant tissue sites (Murungi et al., 2018; Yang et al., 2019).

However, previous studies on insect olfaction have revealed that only a minority of the components in complex odor blends can be detected by insect antennae (Jonsson et al., 1999; Baur et al., 1993; Rojas, 1999). Among the EAG-active compounds, a few appear to be involved in mediating insect behavioral responses (Fraser et al., 2003; Būda et al., 2022). In general, the strength of the EAG response increases with the concentration tested over a range of doses, which is determined by the innate properties of olfactory neurons (Yves et al., 1995; Hoffmann et al., 2020). The differing behavioral effects of different concentrations of compounds are associated with their inherent chemical nature (Liu et al., 2022). The EAG response of the insects only indicated the sensitivity of *B. cucurbitae* to volatile substances, and the specific function of the volatile substances was determined through behavioral experiments.

Volatile components at different concentrations may have different ecological functions, and the same substance may exhibit attractive or repellent effects at different concentrations (Moreno et al., 2022; Cao et al., 2022; Song et al., 2021). In the present study, methyl isovalerate, methyl myristate, methyl laurate, and 1-octadecene were found to have both attractive and repellent effects on *B. cucurbitae*. The effect of methyl isovalerate on both sexes changed from attractive to repellent. According to previous reports,

methyl isovalerate in *Fusarium solani* attracts *Xylosandrus compactus* (Egonyu et al., 2018), and repels flies (Henderson et al., 1991). In addition, 1-octadecene is a repellent component of *Spodoptera frugiperda* (Kong et al., 2023) and an attractive component of *M. incognita* (Ding et al., 2023). Previous studies have shown that methyl myristate is attractive to *Varroa destructor* (Liu et al., 2022), *Drosophila melanogaster*, (Keesey et al., 2017; Dweck et al., 2015), and *Drosophila suzukii* (Matsumura) (Tait et al., 2020) but repellent to *Psoroptes ovis* (Hering) (Dunn et al., 2019). Further, methyl laurate has been previously reported as a pheromone in *D. melanogaster* (Keesey et al., 2017; Dweck et al., 2015) that repels *Amblyomma sculptum* (Barrozo et al., 2021) and *Aedes aegypti* (Zhang et al., 2020). In addition, heptanal can be used as an attractant for *Rhipicephalus sanguineus* (López-López et al., 2023), a sex pheromone of *Plodia interpunctella* (Liu et al., 2021), and as a repellent for *Cotesia glomerata* (L.) (Xu et al., 2020). Lastly, 1-decanol is a repellent for fire ants. The combination of volatiles, as opposed to a single active substance, may be the reason for the differing results in behavioral responses (Stübner et al., 2023; Baig et al., 2023). Thus, methyl isovalerate, methyl myristate, 1-octadecene, methyl laurate, heptanal, and 1-decanol could be mixed in certain proportions for further experiments, laying the foundation for the selection of attractants and repellents.

In this study, we found that several volatile compounds could be used as behavioral regulators in melon flies. Field experiments were conducted on volatile mixtures to develop a synthetic bait for melon flies, which laid the foundation for monitoring and effectively controlling *B. cucurbitae* in an environmentally friendly manner.

Declarations

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Conflicts of Interest/Competing Interests None.

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Figures

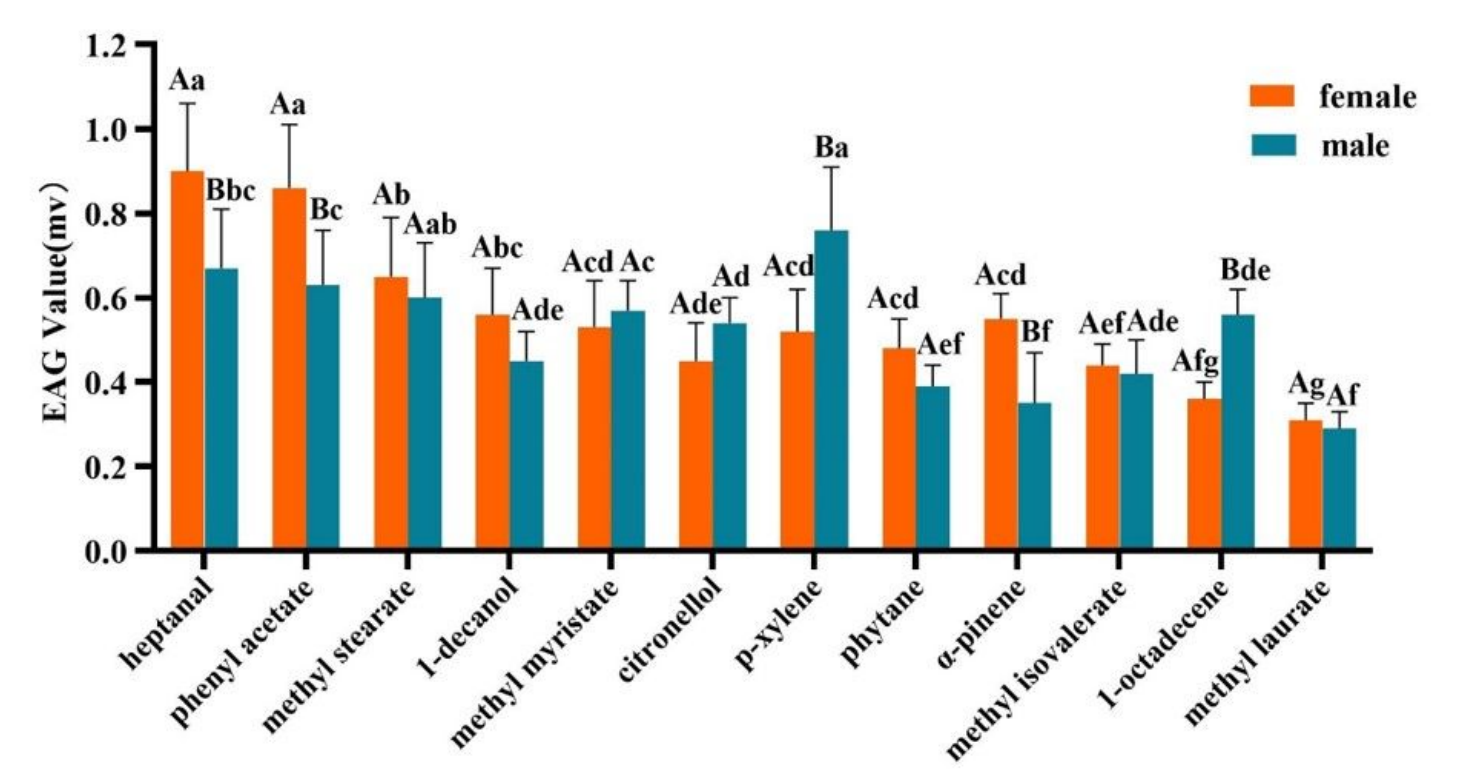


Figure 1

EAG response of *B. cucurbitae* adult females and males to *L. acutangular*. Significant differences between male and female are indicated by capital letter, and significant differences between different odors are indicated by lowercase letters ($P < 0.05$).

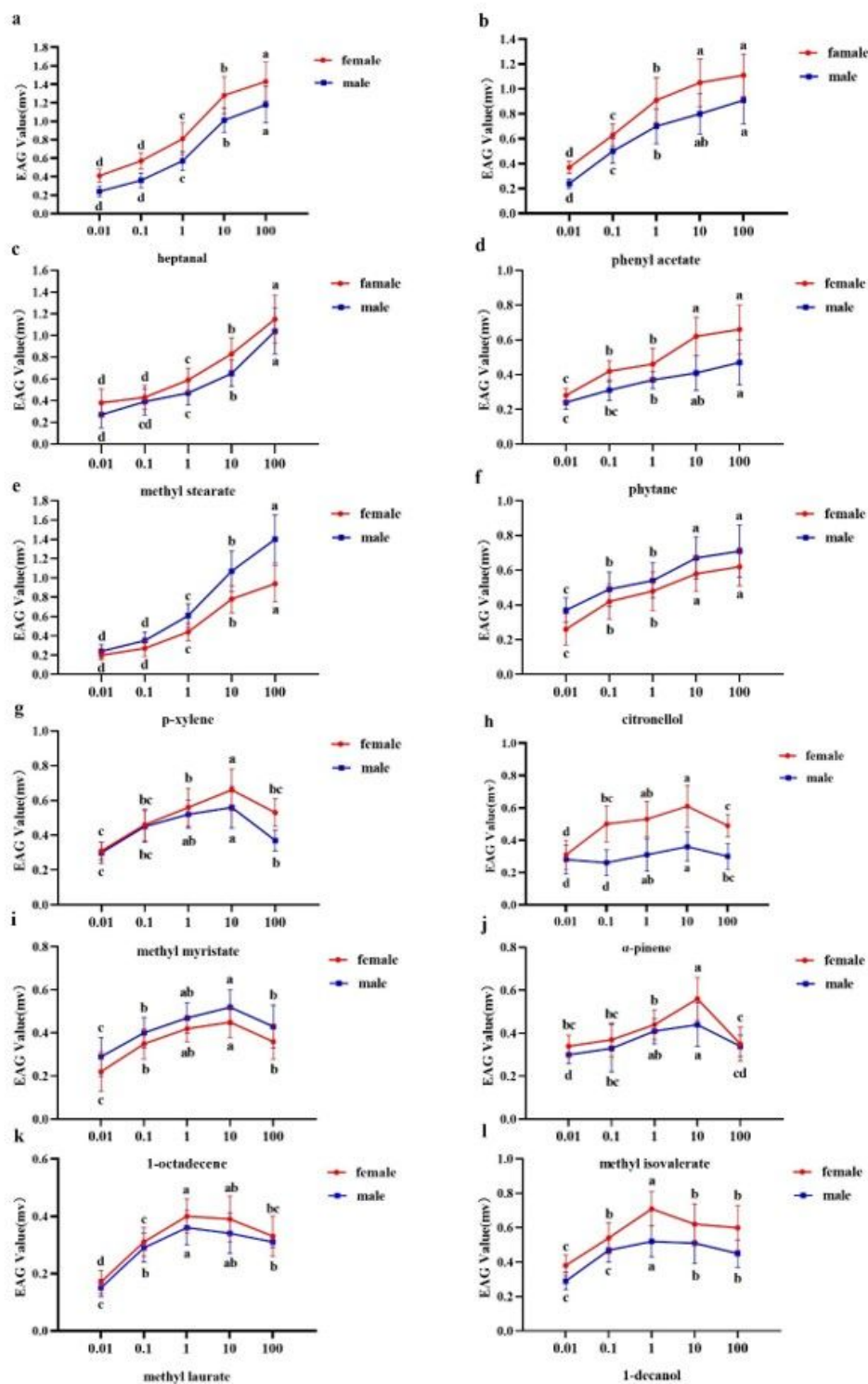


Figure 2

Dose-response of *B. cucurbitae* adult females and males to *L. acutangular*. Significant differences among different doses are indicated by lowercase letters: the same letter means no difference, while different letters mean significant difference (P < 0.05). a: heptanal; b: phenyl acetate; c: methyl stearate; d: phytane; e: p-xylene; f: citronellol; g: methyl myristate; h: α-pinene; i: 1-octadecene; j: methyl isovalerate; k: methyl laurate; l: 1-decanol.

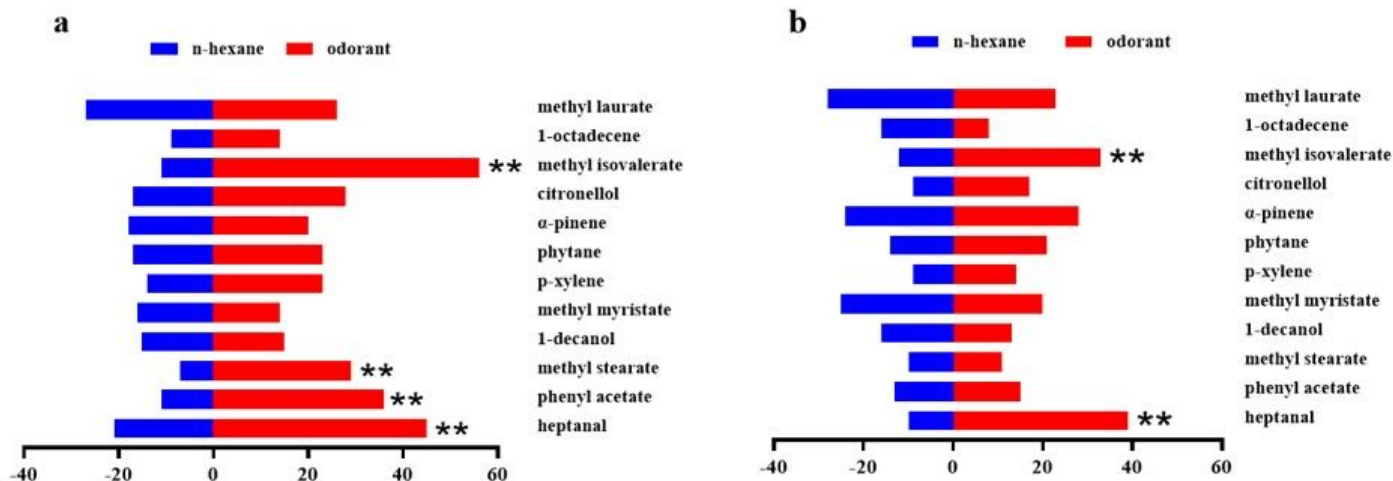


Figure 3

Behavioral responses of *B. cucurbitae* adult females (a) and males (b) (N=100) to 100 µg/µL *L. acutangular*. A positive value of 0 on the right represents attractive effect, and a negative value on the left represents repellent effect. * indicates significant difference at the $P < 0.05$ level by χ^2 test between the treatment and the control, and ** indicates extremely significant difference ($P < 0.01$). Same applies to Figures 4-7.

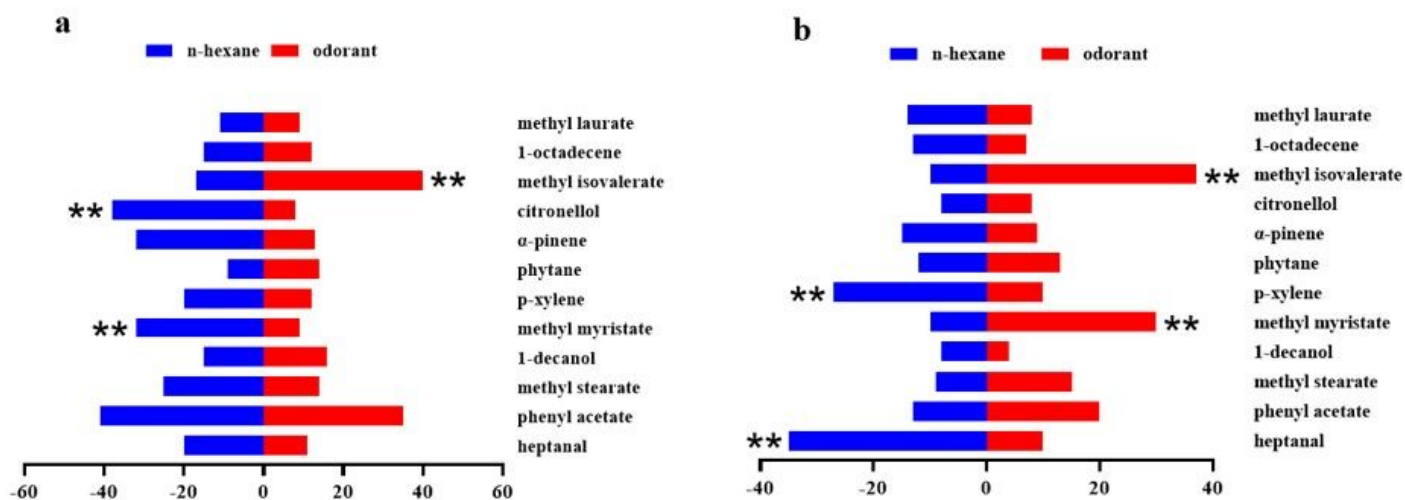


Figure 4

Behavioral responses of *B. cucurbitae* adult females (a) and males (b) (N=100) to 10 µg/µL *L. acutangular*.

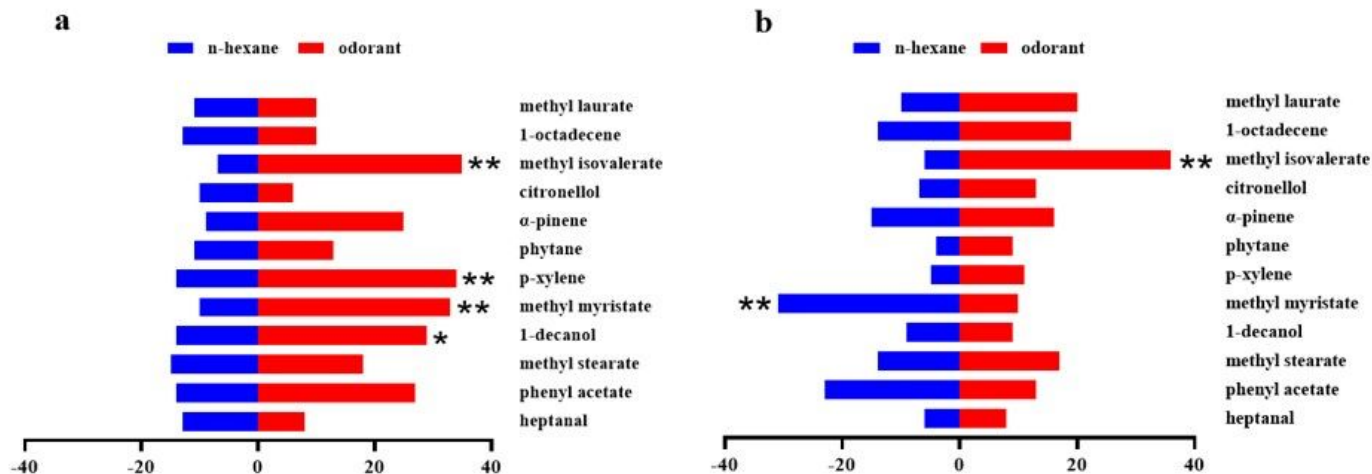


Figure 5

Behavioral responses of *B. cucurbitae* adult females (a) and males (b) (N=100) to 1 µg/µL *L. acutangular*.

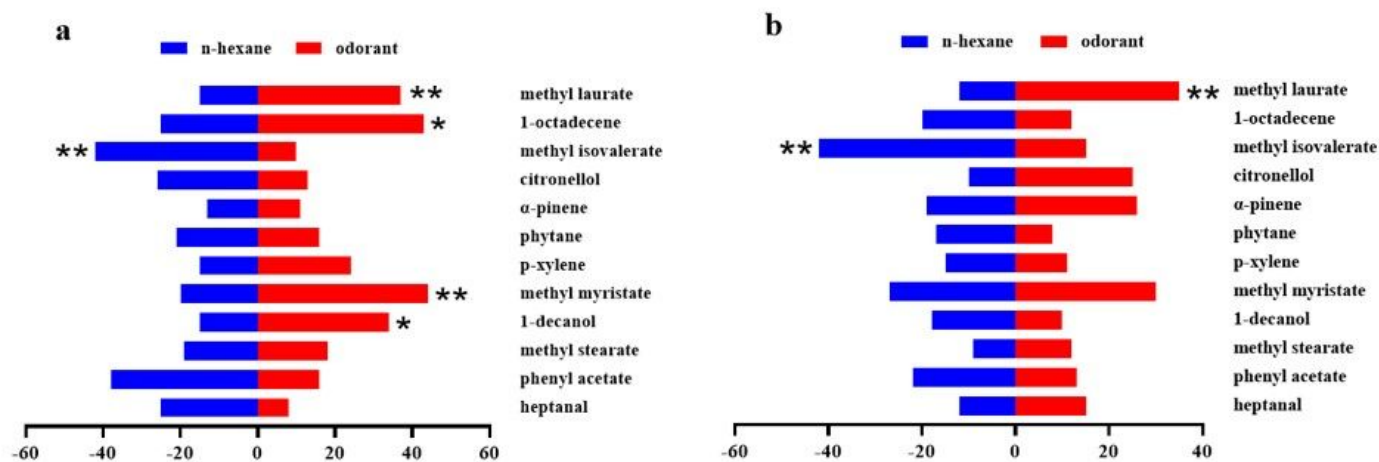


Figure 6

Behavioral responses of *B. cucurbitae* adult females (a) and males (b) (N=0.1) to 0.1 µg/µL *L. acutangular*.

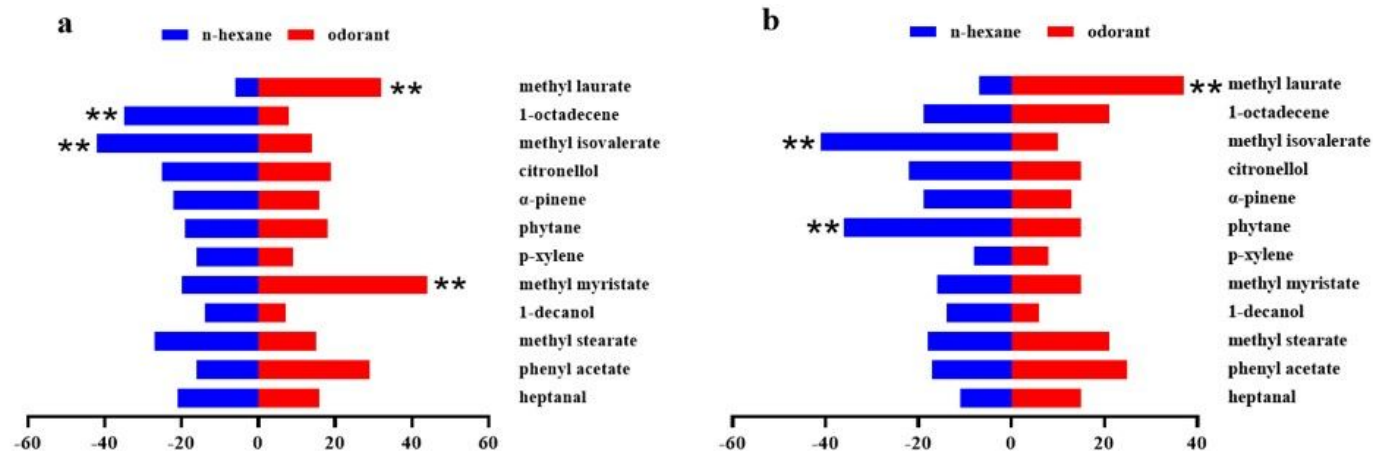


Figure 7

Behavioral responses of *B. cucurbitae* adult females (a) and males (b) (N=100) to 0.01 $\mu\text{g}/\mu\text{L}$ *L. acutangular*.