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Long-chain free fatty acids from *Momordica cochinchinensis* Spreng flowers as allelochemical influencing the attraction of *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae)

A. MUKHERJEE and A. BARIK*

Ecology Research Laboratory, Department of Zoology, University of Burdwan, Burdwan - 713 104, West Bengal, India
E. Mail: anandamaybarik@yahoo.co.in

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ABSTRACT

Extraction, thin layer chromatography and gas chromatography-mass spectrometry of *Momordica cochinchinensis* Spreng (Cucurbitaceae) flowers revealed 13 free fatty acids, and a single flower indicated presence of 364.70 ± 0.04 µg free fatty acids. Myristic and tridecanoic acids were the predominant and least abundant free fatty acids, representing for 97.38 ± 0.76 and 4.86 ± 0.01 µg in a single flower, respectively. The free fatty acids from flowers at 1, 2, 4, 6, 8, and 10 µg concentrations and synthetic blends of fatty acids mimicking different concentrations of flower free fatty acids elicited attraction of female *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae) between 1-10 µg concentrations in Y-shaped glass tube olfactometer bioassay under laboratory condition. The individual synthetic fatty acids mimicking the proportions of fatty acids detected in flowers at different concentrations were also evaluated. Individual synthetic myristic, palmitoleic, *α*-linolenic, and nonadecanoic acids at the minimal amounts of 2.67, 0.56, 0.11, and 0.99 µg, respectively, elicited attraction of the insect. A synthetic blend of 1.60, 0.56, 0.17, and 0.59 µg of myristic, palmitoleic, *α*-linolenic, and nonadecanoic acids, respectively, was most attractive to the insect, and hence, this combination might be used for insect pest management programme such as baited traps.

Keywords: Allelochemicals, *Aulacophora foveicollis*, flower, Free fatty acids, *Momordica cochinchinensis,* Y-tube olfactometer bioassay.

INTRODUCTION

During recent decades, *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae) has become one of the important insect pest of cucurbit crops such as [*Cucurbita maxima* Duchesne*, C. moschata* Duchesne*, C. pepo* L.*., Cucumis sativus* L.*., Lagenaria vulgaris* Ser.*., Luffa cylindrica* L.*., Momordica cochinchinensis Spreng*, and *Benincasa hispida* Thumb.*,* etc. (10,18,23,32)]. Both the larvae and adults cause serious damage to *M. cochinchinensis* (27). Neonate larvae feed on young and healthy roots of this plant for 11-12 days to complete larval development before pupation in the soil, whereas emerged adults consume leaves and flowers voraciously for 8-9 weeks until death. The heavy infestation by this insect causes death of branches and shoots of this plant, which

*Corresponding author

results in reduction of crop production (27). Control of this insect pest may be done through biocontrol agents such as tachinid fly *Medinodexia morgani* (Hardy), mite *Histiostoma* sp., and reduviid bug *Rhinocoris fuscipes* Fabr. (34). The biocontrol programme is not yet successful, and growers are often forced to apply chemical based insecticides such as Carbofuran and Diazinon-60EC to control outbreaks of this insect (10,17,28). There is an increased amount of research evidence that indicates allelochemicals involved in host location may contribute to novel and sustainable pest management programme such as baited traps (19,30,33).

Many compounds (i.e., alkanes, monoterpenes, sesquiterpenes, phenyl propanoids, fatty acids, etc.) serve as olfactory cues for the insects to find their host (26). Long-chain fatty acids affect the behavioral responses in numerous insect species (4,8,12,14,15,20). Further, fatty acids also play major role as precursors in biosynthesis of pheromones (16), structural components of biological membranes (31) and provide energy during the non-feeding stage (6,7). Ectoparasitic mite (*Varroa Jacobsoni* Oud) showed positive response to palmitic acid (20). A mixture of synthetic linoleic, oleic and stearic acids present in maize seedlings extract, attracted the cotton root worm larvae [*Diabrotica virigifera virigifera* (Coleoptera: Chrysomelidae)] (8). Linoleic and linolenic acids, alone and in combination have been shown to attract grasshoppers (4). A synthetic blend of fatty acids, lauric, palmitic, stearic, and oleic acids in the same proportions as present in clover [*Trifolium pratense* (Fabaceae)] root extract attracted female *Hylastinus obscurus* (Coleoptera: Curculionidae) (12). Further, a synthetic blend of palmitic, stearic, oleic, linoleic, and α-linolenic acids mimicking the proportions as present in mature bitter gourd leaves or a synthetic blend of palmitic, stearic and α-linolenic acids mimicking the proportions as present in young bitter gourd leaves attracted female *Epilachna dodecastigma* (Coleoptera: Coccinellidae) (25). Cucurbitacins act as feeding stimulants to *Aulacophora* species (1). A synthetic blend of alkanes in the proportions as present in *M. cochinchinensis* flowers elicited attraction of *A. foveicollis* females (13). As part of our continuous work on bioassay guided isolation and characterization of secondary chemicals from flowers, here we report free fatty acid profile in *M. cochinchinensis* flowers and role of free fatty acids as attractant to *A. foveicollis*. We also studied the role of synthetic blends of fatty acids mimicking the free fatty acids of *M. cochinchinensis* flowers followed by individual synthetic fatty acids and a combination of synthetic fatty acids that elicited response of insects as an olfactory cue for *A. foveicollis*.

MATERIALS AND METHODS

I. Free fatty acid extraction and identification from *M. cochinchinensis* flowers

Momordica cochinchinensis (Cucurbitaceae) plants were cultivated in the field of Crop Research Farm (CRF), The University of Burdwan (23°16′ N and 87°54′ E), West Bengal, India during first week of May 2012, when the environmental temperature was fluctuated between 30- 37°C. The plants were irrigated on alternate days and kept free from insecticide or herbicide use but weeds were removed from the field by hand-picking method weekly. The voucher specimen numbers are Mukherjee and Barik 1 and 2, one of which has been deposited in the Ecotaxonomy Laboratory, Department of Botany, The University of Burdwan, Burdwan, West Bengal, India.

Free fatty acids were extracted by the slightly modified method (3) (Figure 1). One hundred grams of flowers [number of flowers, 68.66 ± 0.66; mean ± Standard error (S. E.)] were dipped in 2 L *n*-hexane for 1 min in room temperature (27 °C), which yielded a straw colored extract, and tricosanoic acid (C23:0) (1 mg) was added to the crude extract as internal standard (IS). The extract was filtered through Whatman No. 41 filter paper, and the solvent was removed under reduced pressure. The dried extract was mixed with diethyl ether and filtered through Whatman No. 41 filter and was purified by TLC on silica gel G (Sigma St. Louis, MO, USA) layers (thickness 0.5 mm), which had been prepared using a Unoplan (Shandon, London) coating apparatus, with *n*-butanol: acetic acid: water (4:1:5; this mixture was shaken and water was separated from this mixture by a separating funnel and discarded, and the final mixture was used as mobile phase) as the mobile phase. The band was eluted from the silica gel layer with diethyl ether, and diethyl ether was removed under reduced pressure to get purified free fatty acids. One portion of the purified free fatty acids was used for olfactory bioassay and another portion for esterification followed by identification through gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS). The purified free fatty acids was esterified with 50 ml CH_3OH: $HClO_4$ (95: 5), and warmed for 10 min in a hot water bath at 50–60°C temperature, and re-extracted three times with 50 ml petroleum ether in a separating funnel (22). The filtrate of each sample was dried over 50 g anhydrous Na_2SO_4, and the esterified fatty acids were initially separated and purified by thin layer chromatography (TLC) using Hexane : Diethyl ether (4: 1) as the mobile phase. The TLC was done on the plate having a silica gel G (Sigma, St. Louis, MO,USA) layer (0.5 mm thickness), which was prepared by using a Unoplan (Shandon, London) coating apparatus (22,25). The single band was eluted from the silica gel layer with petroleum ether, and a portion of each sample was used for GC-FID and another for GC-MS. The extraction of free fatty acids was repeated for three times separately followed by esterification of one portion of each sample.

The purified esterified fatty acids (three separate samples) were analyzed directly by the Techcomp Gas Chromatograph (Em Macau, Rua De Pequim, Nos. 202A-246, Centro Financeiro F7, Hong Kong) model 7900 fitted with a HP-5 capillary column (Agilent, USA; length: 30 m×0.25 mm×0.25-µm film thickness) and a flame ionization detector. Prior to GC, 2 ml petroleum ether was added to each esterified sample every time. The injector port temperature was 280°C. The oven temperature program was initially held at 160°C for 2 min, then raised at the rate of 3°C/ min to 220°C and finally held at 220°C for 18 min. The carrier gas was nitrogen with a flow rate of 20 ml/ min (25). The volume of the sample injected was 1 micro liter with a split ratio of 1: 10. The limit of detection of the GC instrument is $\leq 5 \times 10^{-12}$ g/s (*n*-hexadecane). The peaks were identified by comparison of their retention times with those of standard esterified fatty acids [methyl decanoate (C10:0), methyl laurate (C12:0), methyl tridecanoate (C13:0), methyl myristate (C14:0), methyl pentadecanoate (C15:0), methyl palmitate (C16:0), methyl palmitoleate (C16:1), methyl heptadecanoate (C17:0), methyl stearate (C18:0), methyl oleate (C18:1), methyl linoleate (C18:2), methyl α-linolenate (C18:3), methyl nonadecanoate (C19:0), methyl arachidate (C20:0), methyl heneicosanoate (C21:0), methyl docosanoate (C22:0), methyl tricosanoate (C23:0), and methyl tetracosanoate (C24:0)] (25). The percentage composition of free fatty acids was computed from the GC peak areas and the areas of each peak were converted into quantities of fatty acids based on reference standard methyl

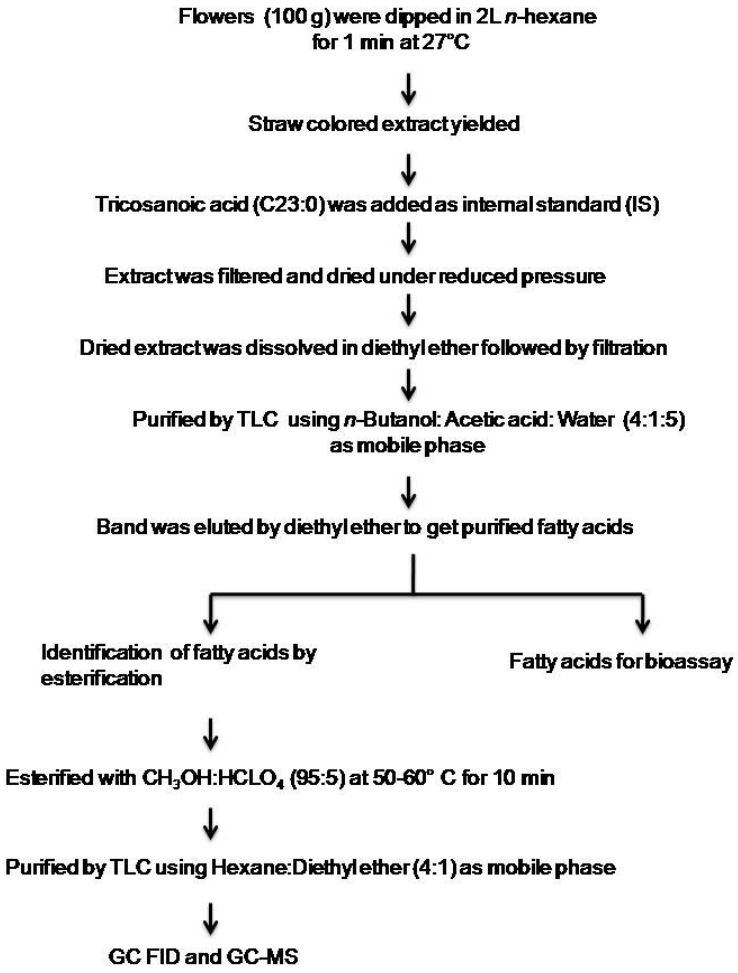

Figure 1. Scheme of free fatty acid isolation and identification.

tricosanoate (C23:0). All solvents used were of analytical grade and purchased from E. Merck (Mumbai, India). All standard esterified fatty acids (fatty acid methyl esters) were purchased from Sigma-Aldrich, Germany.

Another portion of the purified esterified fatty acids were analyzed with an Agilent 6890 GC coupled to a 5973 Mass Selective Detector with a HP-5 column, which was run under same temperature conditions as described for GC analysis. Helium was the carrier gas. The MS temperature parameter was 180°C at the interface, ionization energy 70 eV, scan speed approximately 1 sec, and scanned over the mass range 40–600 mass units. The identity of the compounds was confirmed by injections of mixture of synthetic esterified fatty acids mentioned above. Fatty acids were verified by comparison of the diagnostic ions and GC retention times with those of respective authentic standards.

II. Test insects

The insects used in this study were collected by light trap from the field of The University of Burdwan, where *M. cochinchinensis* plants were growing. The collected insects were maintained in 1 L glass jars, containing *M. cochinchinensis* leaves which were covered with fine-mesh nylon nets at 27±1° C temperature, 65±5% relative humidity (RH) and 12 L: 12 D photoperiod in a biochemical oxygen demand (BOD) incubator (ADS instruments and Tech., Calcutta). *Aulacophora foveicollis* female insects 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 as the adult females consume leaves and flowers of *M. cochinchinensis* plant voraciously for 8–9 weeks until death (27). Females were used in bioassays because they are guided by olfactory cues for both adult feeding and oviposition.

III. Olfactory bioassay

Two mg free fatty acids from *M. cochinchinensis* flowers were dissolved in 20 ml petroleum ether to prepare different concentrations (1, 2, 4, 6, 8, and 10 µg/ml) of free fatty acids blends for olfactory bioassays. The above mentioned concentrations were chosen by preliminary olfactometer trials assuming that a single flower was sufficient to attract the insect, and gradually the dose of the free fatty acids concentrations were lowered until the point where the insect indicated lowest significant response ($P < 0.05$) to the test odor. The highest dose, 10 µg/ml was used in this study because the amounts of fatty acids present in a single flower (364.70 ± 0.04 µg) and 10 µg/ml produced same significant response ($P < 0.00001$) to the test insect. Different concentrations (1, 2, 4, 6, 8, and 10 µg/ml) of synthetic blends of fatty acids mimicking different concentrations of flower free fatty acids blends were prepared (Table 1). The amounts of individual free fatty acids present in different concentrations (1, 2, 4, 6, 8, and 10 µg/ml) of flower free fatty acids were also prepared using synthetic fatty acids for further use in Y-tube bioassay to observe role of individual fatty acids in insect attraction. The standard synthetic fatty acids [i.e., decanoic (C10:0), lauric (C12:0), tridecanoic (C13:0), myristic (C14:0), pentadecanoic (C15:0), palmitic (C16:0), palmitoleic (C16:1), heptadecanoic (C17:0), α-linolenic (C18:3), nonadecanoic (C19:0), heneicosanoic (C21:0), docosanoic (C22:0), and tetracosanoic (C24:0) acids], that were identified in natural flowers, were all purchased from Sigma Aldrich, Germany. The Y-shaped glass tube olfactometer, used in the bioassay has two 0.6 cm radius × 5 cm long arms, and a 0.6 cm radius × 5 cm long common arm (2, 13, 22). The common arm of the Y-shaped glass tube olfactometer was connected to a porous glass vial (1 cm radius × 3 cm long) in which test insect was released for the olfactory bioassay. The right and left arm of the Y-tube were connected to two glass made micro kit adapters which were fitted into two 1 cm radius × 3 cm long glass vials. One glass vial contained a piece (2 cm^2) of Whatman No. 41 filter paper moistened with 1 ml of a particular concentration of free fatty acids (0.5 ml of fatty acid sample was first applied on the filter paper followed by evaporation at open space under laboratory condition for 30 sec and again 0.5 ml of fatty acid sample was again applied on the same filter paper), whilst the other glass vial contained a piece (2 cm^2) of Whatman No. 41 filter paper moistened with 1 ml of the control solvent (petroleum ether). Each membrane pump

Table 1. Proportions of different synthetic fatty acids mixed to prepare concentrations mimicking free fatty acids of *M. cochinchinensis* flowers

Synthetic fatty acid	Concentration (µg)					
	1	2	4	6	8	10
Decanoic acid (C10:0)	0.04	0.09	0.18	0.27	0.36	0.45
Lauric acid (C12:0)	0.05	0.09	0.19	0.28	0.38	0.47
Tridecanoic acid (C13:0)	0.01	0.03	0.05	0.08	0.11	0.13
Myristic acid (C14:0)	0.27	0.53	1.07	1.60	2.14	2.67
Pentadecanoic acid (C15:0)	0.02	0.04	0.08	0.12	0.16	0.20
Palmitoleic acid (C16:1)	0.09	0.19	0.37	0.56	0.74	0.93
Palmitic acid (C16:0)	0.06	0.12	0.24	0.36	0.47	0.59
Heptadecanoic acid (C17:0)	0.07	0.14	0.27	0.41	0.54	0.68
α-Linolenic acid (C18:3)	0.03	0.06	0.11	0.17	0.22	0.28
Nonadecanoic acid (C19:0)	0.10	0.20	0.39	0.59	0.79	0.99
Heneicosanoic acid (C21:0)	0.05	0.10	0.19	0.29	0.38	0.48
Docosanoic acid (C22:0)	0.05	0.10	0.21	0.31	0.42	0.52
Tetracosanoic acid (C24:0)	0.16	0.32	0.65	0.97	1.3	1.62

produced an air-flow of 450 ml min^{-1}, were first purified by passing through charcoal pellets and then led into left and right glass vials. All the connections between different parts of the set-up consisted of silicon tubing.

The effectiveness of fatty acids as attractants was evaluated in the following manner in the laboratory at 27±1ºC, RH 70±3 %, and light intensity 150 lux. One ml free fatty acids (0.5 ml of fatty acid sample was first applied on the filter paper followed by evaporation at open space under laboratory condition for 30 sec and again 0.5 ml of fatty acid sample was again applied on the same filter paper) from each concentration (1, 2, 4, 6, 8, and 10 µg/ml) and the pure solvent were applied to the filter paper pieces and allowed to evaporate the solvent in open space under laboratory condition, and these filter papers were introduced into the glass vials. One adult female, *A. foveicollis* was introduced into a porous glass vial (1 cm radius × 3 cm long) which was then attached with the common arm of the olfactometer, and exposed to a particular odor, consisting of 1 ml of the control solvent (petroleum ether) in one glass vial, and 1 ml of one of the different odors (free fatty acids) in another glass vial. This insect was not attracted by the control solvent in preliminary assays. The behaviour of insects, i.e., olfactory responses of insects toward fatty acid odor air flowing through one arm and control solvent air flowing through other arm was studied in a Y-tube olfactometer for 30 min in preliminary bioassays, and subsequently, it was observed that olfactory responses of the insects either in the fatty acid odor-loaded arm or control solvent-loaded arm at 3 min and 30 min were same. Hence, the behavior of each female was observed for 3 min in the Y-tube in all further bioassays. A female was considered to have made a choice if it entered either arm, and when the test insect reached the end of the odor-loaded arm or solvent-loaded arm of the olfactometer, the insect was removed from the Y-tube, and the choice of the insect was recorded as a positive response or negative response by one unit, respectively. By contrast, a female was considered not having made a choice, i.e., "no response" if it remained in the common arm of the Y-tube by the end of the observation period, because only the scores for odor loaded air-flow or control solvent air-flow can be compared with each other (11,13,22,24,25).

Each experiment with one free fatty acid sample was conducted with a group of 90 näive insects; and after testing 5 insects the olfactometer set-up was cleaned with petroleum ether followed by acetone, and the position of the two arms was systematically changed in order to avoid positional bias. The positive or negative responses of 90 individual naïve insects were taken excluding the number insects that did not produce any choice to the fatty acid odor or control solvent. Experiments with flower free fatty acid samples, synthetic blends of fatty acids, and individual synthetic fatty acids were conducted in the same manner. Around 10 % of adult females in each concentration remained unresponsive and they were excluded from the analyses.

Statistical analyses

The data obtained on responses of *A. foveicollis* to different concentrations of free fatty acids obtained from *M. cochinchinensis* flowers, synthetic blends of fatty acids, and individual synthetic fatty acids were analyzed based on the null hypothesis that the probability of scores for the test compound(s) or control solvent is equal to 50 %, i.e., Chi-square analysis (11,13,22,24,25).

RESULTS AND DISCUSSION

One hundred grams of *M. cochinchinensis* flowers yielded 25.04 ± 0.05 mg of purified free fatty acids; whereas a single flower contained 364.70 ± 0.04 µg (± represents S. E.) of free fatty acids. The GC-FID and GC-MS analysis of free fatty acids of *M. cochinchinensis* flowers revealed 11 saturated and 2 unsaturated free fatty acids (Table 2) (Figure 2). Myristic acid (C14:0) was the major free fatty acid representing for 97.38 ± 0.76 µg, whereas tridecanoic acid (C13:0) was the least abundant (4.86 ± 0.01 µg) fatty acid in a single flower. Tetracosanoic acid (C24:0) is the second most abundant fatty acid followed by nonadecanoic (C19:0), palmitoleic (C16:1), heptadecanoic (C17:0), palmitic (C16:0), docosanoic (C22:0), heneicosanoic (C21:0), lauric (C12:0), decanoic (C10:0), *α*-linolenic (C18:3), and pentadecanoic (C15:0) acids, accounting for 59.16 ± 1.19, 35.95 ± 0.39, 33.85 ± 0.65, 24.74 ± 0.11, 21.60 ± 0.13, 19.05 ± 0.17, 17.33 ± 0.44, 17.14 ± 0.29, 16.29 ± 0.95, 10.04 ± 0.12, 7.31 ± 0.27 µg, respectively. The relative amounts of fatty acids found in aril and seeds of this plant displayed different distribution patterns, and oleic (C18:1) and stearic (C18:0) acids were the major fatty acids in aril and seeds of *M. cochinchinensis* fruit, respectively (9). However, this study revealed that myristic acid was the major free fatty acid followed by tetracosanoic acid in flowers of *M. cochinchinensis* plant. Saturated fatty acids were higher in *Fuchsia fuelgens* leaf (21), and *Abies pindrow* (5) belonging to Onagraceae and Pineceae family, respectively, and this study also demonstrated higher amounts of saturated fatty acids than unsaturated fatty acids in *M. cochinchinensis* flowers.

Olfactometric bioassay tests examining the effectiveness of free fatty acids isolated from *M. cochinchinensis* flowers at several concentrations are presented in Table 3. Odorous free fatty acids blends from *M. cochinchinensis* flowers attracted *A. foveicollis* at 1, 2, 4, 6, 8, and 10 µg concentrations with 62, 69, 72, 79, 83, and 88 % insects

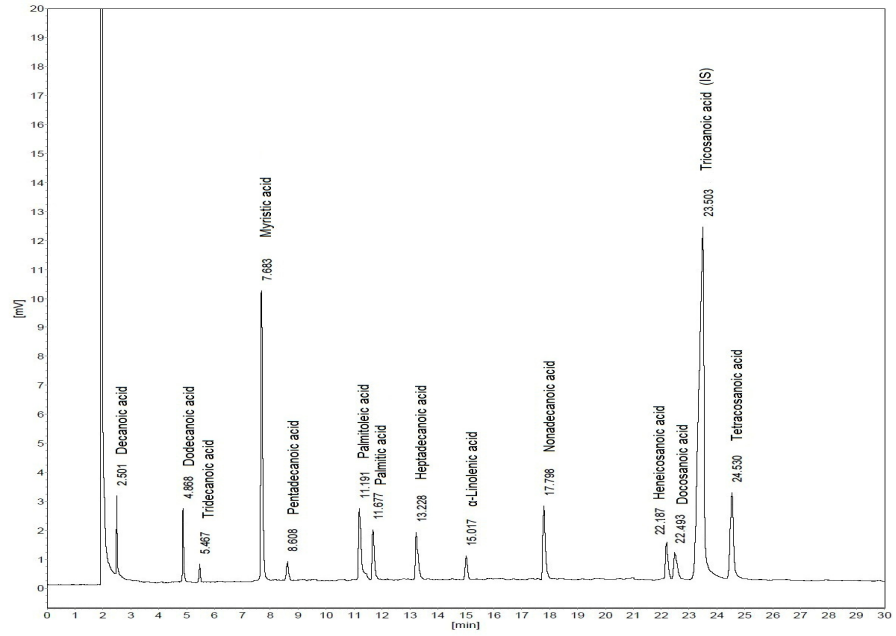

Figure 2. Example GC-FID chromatogram (HP-5) of free fatty acids detected in *M. cochinchinensis* flowers.

Table 2. Free fatty acids in a single flower of *M. cochinchinensis* plant (mean ± SE, *N* = 3)

Fatty acid	Amount (µg)
Decanoic acid (C10:0)	16.29 ± 0.95
Lauric acid (C12:0)	17.14 ± 0.29
Tridecanoic acid (C13:0)	4.86 ± 0.01
Myristic acid (C14:0)	97.38 ± 0.76
Pentadecanoic acid (C15:0)	7.31 ± 0.27
Palmitoleic acid (C16:1)	33.85 ± 0.65
Palmitic acid (C16:0)	21.60 ± 0.13
Heptadecanoic acid (C17:0)	24.74 ± 0.11
α-Linolenic acid (C18:3)	10.04 ± 0.12
Nonadecanoic acid (C19:0)	35.95 ± 0.39
Heneicosanoic acid (C21:0)	17.33 ± 0.44
Docosanoic acid (C22:0)	19.05 ± 0.17
Tetracosanoic acid (C24:0)	59.16 ± 1.19

Table 3. Attractiveness of *M. cochinchinensis* flower free fatty acids odour and synthetic blends of fatty acids mimicking free fatty acids of flower to *A. foveicollis* choosing odour arm (%) in the Y-shaped glass tube olfactometer bioassay (*N* = 90 in each bioassay)

Fatty Acid	Concentration (µg)					
	1	2	4	6	8	10
Natural	62*	69**	72***	79****	83*****	88*****
Synthetic	61*	68**	71***	77****	81*****	87*****

*, **, ***, and **** indicate significant responses at the level of 0.05, 0.001, 0.0001, and 0.00001, respectively. For preparation of mixture of synthetic fatty acids see Table 1.

Olfactory bioassay experiments of *A. foveicollis* toward the amounts of individual synthetic fatty acids mimicking individual free fatty acids present in flowers at different concentrations revealed that myristic, palmitoleic, *α*-linolenic, and nonadecanoic acids attracted the insect (Table 4). Application of 2.67 µg of myristic acid present in 10 µg fatty acids concentrations of flowers attracted 67 % insects. Adult females were attracted to 0.56, 0.74, and 0.93 µg of synthetic palmitoleic acid present in 6, 8, and 10 µg free fatty acids concentrations with 62, 69, and 78 % insects responding, respectively. *α*-Linolenic acid present at 0.11, 0.17, 0.22, and 0.28 µg in 4, 6, 8, and 10 µg concentrations of flower free fatty acids elicited attraction of 64, 70, 74, and 79 % insects, respectively. Nonadecanoic acid at 0.99 µg attracted 67 % insects; whereas rest of the identified fatty acids present in different amounts at different concentrations of flower free fatty acids did not provoke any response to the insect. The synthetic blends of 4 fatty acids attracted 62, 69, 74, 79, and 83 % of the insect, *A. foveicollis* at the amount of equivalent flower fatty acid of 2, 4, 6, 8, and 10 µg, respectively (Table 4).

Table 4. Attractiveness of individual synthetic fatty acids mimicking the amounts of individual free fatty acids and their combinations detected in free fatty acids of *M. cochinchinensis* flowers to *A. foveicollis* choosing odor arm (%) in the Y-shaped glass tube olfactometer (N = 90 in each bioassay)

Synthetic fatty acid	Concentration (µg)					
	1	2	4	6	8	10
C14:0 (A)	n. r.[a] (0.27)[c]	n. r. (0.53)	n. r. (1.07)	54 (1.60)	60 (2.14)	67**[b] (2.67)
C16:1 (B)	n. r. (0.93)	51 (0.19)	56 (0.37)	62* (0.56)	69*** (0.74)	78**** (0.93)
C18:3 (C)	53 (0.03)	59 (0.06)	64** (0.11)	70*** (0.17)	74**** (0.22)	79**** (0.28)
C19:0 (D)	n. r. (0.10)	n. r. (0.20)	n. r. (0.39)	n. r. (0.59)	58 (0.79)	67** (0.99)
A + B + C + D	57	62*	69***	74****	79****	83****

a : No response. b: *, **, ***, and **** indicate significant responses at the level of 0.05, 0.01, 0.001, and 0.00001, respectively. c: The actual amount (µg) of each fatty acid represented in the bioassay.

The present olfactory bioassay results clearly demonstrated that long-chain free fatty acids attracted the insect, *A. foveicollis,* which are low volatile substances that might act as close range allelochemicals during flowering of the plant. Long-chain fatty acids as allelochemicals have been reported to affect behavioral responses in a number of insect species (4,8,12,14,15,20,29). A single flower possessed 364.70 ± 0.04 µg free fatty acids, suggesting that a single flower is attractive to female *A. foveicollis*, as 10 µg free fatty acids from flowers attracted 88 % ($P < 0.00001$) of the insects.

In the present study, free fatty acids isolated from *M. cochinchinensis* flowers and the synthetic blends of fatty acids mimicking flower free fatty acids attracted the test insect, *A. foveicollis* between 1–10 µg concentrations. Olfactory bioassay experiments with the individual synthetic fatty acids mimicking the proportions present in different concentrations of flower fatty acids indicated that myristic, palmitoleic, *α*-linolenic, and

nonadecanoic acids were responsible for attraction of the insect, and a synthetic blend of the above mentioned 4 fatty acids indicated that attraction of the insect toward this combination was higher due to synergistic effect of the individual fatty acids. A synthetic blend mimicking free fatty acids combinations and proportions detected in flowers at 6 µg concentration and a synthetic blend of 4 fatty acids at the amount of equivalent flower fatty acids of 6 µg concentration produced same responses ($P < 0.00001$) to the insect. Further, a synthetic blend of myristic (1.60 µg), palmitoleic (0.56 µg), α-linolenic (0.17 µg), and nonadecanoic (0.59 µg) acids at 6 µg concentration attracted 74 % ($P < 0.00001$) of the insects or a synthetic blend of myristic (2.67 µg), palmitoleic (0.93 µg), α-linolenic (0.28 µg), and nonadecanoic (0.99 µg) acids showed attraction of 83 % ($P < 0.00001$) *A. foveicollis*, demonstrating that a synthetic blend of former combination of myristic, palmitoleic, α-linolenic, and nonadecanoic acids might be used during flowering of crops for insect pest management programme such as baited traps. This study reveals that *A. foveicollis* elicited attraction to free fatty acids as short-range volatiles which are perceived by their olfactory sensilla located on the antennae and employs these cues in host location. But bioassays in the greenhouse to evaluate responses of *A. foveicollis* to a synthetic blend of 4 fatty acids at an amount equivalent to 6 µg of the flower free fatty acids are needed further to study the effectiveness of volatility of the fatty acid compounds as attractant tested in the present study.

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