



Synergism in Host Selection Behavior of Three Generalist Insects Towards Leaf Cuticular Wax of Sesame Cultivars

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Abstract

Leaf cuticular wax plays important role in host selection, oviposition, and feeding of phytophagous insects. Thus, the role of cuticular wax of sesame (*Sesamum indicum*) cultivars (Savitri and Nirmala) in host selection of 3 generalist pests (*Spilosoma obliqua* Walker, *Helicoverpa armigera* Hübner, and *Spodoptera litura* Fabricius) was investigated under laboratory conditions. The GC-MS and GC-FID analyses of leaf surface waxes of both cultivars indicated the presence of 14 n-alkanes from n-C₉ to n-C₄₄ and 12 free fatty acids (FFAs) from C_{9:0} to C_{20:0}. The most predominant n-alkane and FFA of the cultivars were n-C₂₆ (94.3 ± 7.27 µg leaf⁻¹) and C_{18:1} (110.8 ± 10.07 µg leaf⁻¹), respectively present in Savitri cultivar. The generalists used visual (color and shape), olfactory (odorous n-alkanes and FFAs), tactile (surface ultra-structure), and gustatory (cuticular wax) cues in a synergistic manner for their host selection through attraction (adults and larvae) followed by oviposition (adults) and feeding (larvae) on studied cultivars (Savitri > Nirmala). Their olfactory responses were maximum towards 2 leaf equivalent amount, whereas oviposition and feeding preference were maximum towards 4 leaf equivalent amount of the combined synthetic (4 n-alkanes (n-C₁₆, n-C₂₂, n-C₂₄, n-C₂₆) + 3 FFAs (C_{12:0}, C_{14:0}, C_{18:1})) mixture-treated intact leaf of cultivar Savitri. This finding can suggest that the synthetic blend (4 n-alkanes + 3 FFAs) in leaf equivalent amount (396.6 ± 4.13 µg leaf⁻¹) or more from cultivar Savitri can be used as lures to develop baited trap. In addition, the cultivar Nirmala can be used as a resistant cultivar in the ecological pest management (EPM) framework of these target pest species.

Keywords *Sesamum indicum* · *Spilosoma obliqua* · *Helicoverpa armigera* · *Spodoptera litura* · n-alkanes · free fatty acids

Introduction

In the new era of chemical ecology, isolation and identification of semiochemicals responsible for insect behavior enhance a better understanding of insect-plant interactions (Schoonhoven et al. 2005; Little et al. 2019). Generally, herbivore insects recognize their host plants by several physicochemical cues through different sensory modalities (Renwick and Chew 1994; Lucas-Barbosa et al. 2016; Roy 2019a). The first physical contact between a defoliator insect and host plant occurs on the leaf cuticular surface and acts as low volatile cues for host acceptance (Jetter and Schäffer 2001, Jetter et al. 2006, Das et al.

2019, Fernández et al. 2019). Thus, leaf surface wax plays important role in short-range attractant for host recognition, oviposition, and feeding stimulant in different phytophagous insects (Li and Ishikawa 2006; Mitra et al. 2017, 2019, 2020). Particularly, it is very crucial in lepidopterans because their neonates are often relatively immobile and, thus, depend on the judicious choice of the host plant by females (Müller and Riederer 2005; McCallum et al. 2011). The preference-performance hypothesis (PPH) or “mother-knows-best” similarly states that natural selection favors those insect females which prefer host plants where the offspring performs best, especially when immature stages are less mobile than adults (Gripenberg et al. 2010; Altesor and González 2018; Birke and Aluja 2018; Griese et al. 2020). Even, for a polyphagous pest, a broader diet increases the risk of oviposition on non-host or poor host along with evaluation time due to limitations in the extraction of information from host volatiles from the noise of non-host volatiles in an ecological context (Lucas-Barbosa et al. 2016).

The leaf surface wax consists of long-chain alkanes, free fatty acids (FFAs), esters, aldehydes, and primary and secondary alcohols, which vary widely within species or cultivars of

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a species (Jetter et al. 2006; Roy et al. 2012a; Roy and Barik 2012b; Mitra et al. 2020). The importance of plant leaf alkanes and FFAs as allelochemicals has been demonstrated using different insects in the last two decades (Roy and Barik 2014; Roy et al. 2012b; Roy 2019a; Mitra et al. 2020; Mobarak et al. 2020a). Especially, low volatile n-alkanes and FFAs serve an important role in insect-plant interactions like olfactory attractant (Roy et al. 2012a; Roy and Barik 2012b, 2014; Karmakar et al. 2016; Malik et al. 2017; Mobarak et al. 2020a) and/or oviposition stimulant (Eigenbrode and Espelie 1995; Parr et al. 1998; Li and Ishikawa 2006; Mitra et al. 2017). Thus, plant surface texture and chemistry are the most important source of information for moths in the evaluation of their potential oviposition sites (Renwick and Chew 1994; Potter et al. 2012; Späthe et al. 2013). There are a handful of studies that investigate attraction to host plant volatiles (Ikeura et al. 2010; Mitra et al. 2019, 2020; Mobarak et al. 2020a) but unequivocal evidence for host physicochemical cues used in orientation and oviposition site selection by lepidopterans has so far been scarce. Even, there is currently no information about physicochemical cues mediated attraction, oviposition, and feeding preference in integration to any insect-plant interactions except the previous report on *Diacrisia casignetum* Kollar (Lepidoptera: Arctiidae) (Roy 2019a). Thus, this behavioral study on other such generalist pests like *Spilosoma obliqua* Walker (Lepidoptera: Arctiidae), *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), and *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) on sesame (*Sesamum indicum* L., Pedaliaceae) cultivars (Savitri and Nirmala) are immensely important for monitoring these pests along with their management.

These primary generalist (polyphagous) pest species (*S. obliqua*, *H. armigera*, and *S. litura*) are responsible for causing damage to a variety of other economically important crop plants (Xue et al. 2010; Roy and Barik 2012a; Gotyal et al. 2015; Basavaraj et al. 2018; Roy 2020). The most damaging stage of these pest species are their middle to mature age caterpillars (3rd–5th instars) and which feeds gregariously on their host plants (Liu et al. 2004; Xue et al. 2010; Roy and Barik 2013; Mobarak et al. 2020b; Roy 2020, 2021). Even, their larvae showed certain levels of resistance to a different class of insecticides, and hence, successful control of these pests is to some extent difficult (Mohapatra and Gupta 2018; Roy 2019b, 2020). The uses of several botanicals have already attained the status of potential pesticides in place of synthetic insecticides against them (Singh et al. 2007; Kumar and Ali 2010). There is an alternate safe strategy for limiting herbivore by the selection of high-yielding resistant varieties against their pests (Wolfenbarger and Phifer 2000; Mobarak et al. 2020b). Consequently, a comprehensive behavioral study of these pests on most cultivated sesame cultivars (Savitri

and Nirmala) is needed to promote integrated pest management (IPM) and to reduce the reliance on chemical pesticides.

In view of the potential for using host-derived semiochemicals in insect pest management, the aims of this study were (i) to identify and quantify the composition of leaf cuticular wax chemicals (n-alkanes and FFAs) present in two widely used cultivars (Savitri and Nirmala) of sesame due to their high yielding potential; (ii) to evaluate the role of respective leaf surface wax chemicals followed by their synthetic analogs and their mixtures (n-alkanes and FFAs) in short-range attraction, oviposition, and feeding of three generalist pests (*S. obliqua*, *H. armigera*, and *S. litura*) through different bioassay experiments under laboratory conditions; and (iii) to find out the most effective combination of wax chemicals (n-alkanes and FFAs) in attraction, oviposition, and feeding of the generalist pests for designing a baited trap as well as more resistant cultivar of sesame as a part of IPM in near future.

Materials and methods

Plants

Two widely used cultivars (Savitri and Nirmala) of sesame (*S. indicum*) were cultivated in a selected field in West Bengal, India (22°53'N, 88°23'E, 13 m above sea level) during 2019–2020 growing season. Six plots (each plot 10 m × 10 m; soil organic matter 5.3 ± 0.2%, pH 7.7, average relative humidity (RH) 70 ± 15%, average photoperiod 13 L:11 D at 30–36°C) were prepared for the cultivation of the sesame cultivars with an average plant density of 30 ± 2 plants m⁻². The selected cultivars were separately germinated and each was grown in three side by side plots with a gap of 0.5 m between two plots. A space of 1 m was kept for the cultivation of another cultivar and all plots were maintained without any insecticide spraying.

Two to three mature leaves were collected from each 6–8 week old plant at 6 AM. Three separate batches of around 100-g leaves of each cultivar were collected from the respective plots for extraction of leaf surface waxes. Only mature leaf surface waxes of sesame cultivars (Savitri and Nirmala) were considered during this study because polyphagous herbivores generally prefer to feed on host mature leaves.

Insects

Adults of three generalist pests (*S. obliqua*, *H. armigera* and *S. litura*) were collected by light trap from jute crops (*Corchorus capsularis*, cultivar: Sonali (JRC-321), Malvaceae) in West Bengal, India. After collection, the specimens were disposed of on the same jute leaves separately for egg laying in oviposition cages. Newly emerged first instar

larvae (F_1) of each pest species were placed separately on the same jute leaves for feeding and they were kept at $27 \pm 1^\circ\text{C}$, $70 \pm 10\%$ RH, and 12 L: 12 D photoperiod with light intensity of 1500 lux in a Biological Oxygen Demand (BOD) incubator as in Roy (2019a, 2019b, 2020).

Three generations (F_1 – F_3) of each pest species were completed on jute leaves. The F_4 females (1–2 days old) of each species were used for olfactory and oviposition bioassays as well as fourth instar F_4 larvae (9–12 days old) were used for attraction and feeding bioassays in laboratory conditions. The larvae (4th instar) and adults (females) of *S. obliqua*, *H. armigera*, and *S. litura* were not reared on the sesame cultivars (Savitri and Nirmala) other than jute based on Hopkins' host-selection principle (Barron 2001) to avoid any biasness to leaf surface waxes of sesame cultivars during their bioassays.

Extraction of leaf surface wax

Freshly collected mature sesame leaves of about 100 g for each cultivar (fresh weight 1.6 ± 0.11 and 1.4 ± 0.08 g leaf⁻¹ (mean \pm SE) for Savitri and Nirmala, respectively (SM – Table 1)) were dipped in 2 L n-hexane separately for 1 min at room temperature for extraction of their surface wax which yielded straw-colored extracts without a trace of any chlorophyll (Roy et al. 2012a; Mitra et al. 2020). The crude extract was passed through filter paper (Whatman No. 41, Whatman International Ltd., Maidstone, England) and was evaporated at room temperature (27°C) to dryness. The extraction was

repeated three times separately for each cultivar and the dry extract (wax) yields were 43.034 ± 0.931 and 38.460 ± 0.466 mg 100 g⁻¹ in cultivars Savitri and Nirmala leaves, respectively (SM – Table 1).

Each crude extract was then dissolved in 40 ml n-hexane and divided into four equal portions (equivalent to 25 g of leaves); the first one was used for identification and quantification of n-alkanes and FFAs, whereas the remaining second, third, and fourth ones after purification were used for attraction, oviposition, and feeding bioassays, respectively. All solvents used were of analytical grade and purchased from E. Merck (E. Merck Ltd., Mumbai, Maharashtra, India). All standard n-alkanes and fatty acids (FAs) (> 99% purity) were purchased from Sigma-Aldrich (Sigma-Aldrich, Tanfkirchen, Germany).

Analysis of n-alkanes

One-half of the first portion of each crude extract of each kind of sesame leaves (cultivars Savitri and Nirmala) was passed through a column of aluminum oxide (F-20 grade, Alcoa, Frankfurt, Germany) and eluted with petroleum ether. The eluent was fractionated by thin-layer chromatography (TLC) on silica gel G (Sigma St. Louis, MO, USA) of 0.5-mm thickness by using carbon tetrachloride (CCl_4) as the mobile phase. A faint yellowish band was appeared on the TLC plate, and the plate was air-dried under laboratory conditions. The R_f (retention factor) value (0.86) was compared with the R_f -value of a mixture of synthetic n-alkanes between n-C₁₀ and n-C₄₀.

Table 1 Composition of alkanes ($\mu\text{g leaf}^{-1}$) in leaf surface waxes (mean \pm SE, n = 3) of two selected cultivars (Savitri and Nirmala) of sesame (*Sesamum indicum*, Pedaliaceae) determined during their growing season in 2019–2020

Alkanes ($\mu\text{g leaf}^{-1}$)	Savitri	Nirmala	$F_{1,4}$	<i>p</i>
Nonene (n-C ₉)	29.5 \pm 2.27	7.0 \pm 0.57	91.565	< 0.001
Dodecene (n-C ₁₂)	5.5 \pm 0.43	--	168.055	< 0.001
Tetradecane (n-C ₁₄)	14.7 \pm 1.13 ^A	--	168.055	< 0.001
Pentadecane (n-C ₁₅)	3.2 \pm 0.25 ^B	10.6 \pm 0.86	68.152	< 0.001
Hexadecane (n-C ₁₆)	36.6 \pm 2.82	21.5 \pm 1.75	20.692	0.010
Eicosane (n-C ₂₀)	14.5 \pm 1.12 ^A	31.5 \pm 2.56	36.853	0.004
Docosane (n-C ₂₂)	90.4 \pm 6.97	33.9 \pm 2.75	56.842	0.002
Tricosane (n-C ₂₃)	--	12.0 \pm 0.98	151.280	< 0.001
Tetracosane (n-C ₂₄)	19.4 \pm 1.49	44.5 \pm 3.62	41.274	0.003
Pentacosane (n-C ₂₅)	0.9 \pm 0.07	--	168.055	< 0.001
Hexacosane (n-C ₂₆)	94.3 \pm 7.27	25.4 \pm 2.07	82.973	< 0.001
Octacosane (n-C ₂₈)	7.9 \pm 0.61	27.1 \pm 2.20	70.504	< 0.001
Hexatriacontane (n-C ₃₆)	10.1 \pm 0.78	15.9 \pm 1.29	14.705	0.019
Tetratetracontane (n-C ₄₄)	3.4 \pm 0.27 ^B	10.7 \pm 0.87	63.732	< 0.001
Total n-alkanes	290.2 \pm 3.97	249.9 \pm 6.74	26.610	0.007

Note: Within rows and columns means followed by the same letters (lowercase and uppercase, respectively) are not significantly different ($p \geq 0.05$) by Tukey's HSD test

The single hydrocarbon band produced in each TLC plate was eluted from the silica gel layer with chloroform, which showed only C-H stress in IR spectroscopy (JASCO FT-IR spectrophotometer).

The purified alkane samples were used for gas chromatography-mass spectrometry (GC-MS) and GC-FID (flame ionization detector) for identification and quantification, respectively as described by Roy (2019a). The extracts were analyzed with a Shimadzu GCMS-QP5050A to produce electron ionization (EI) mass spectra using HP-5MS column for GCMS-EI analysis by using a specified oven temperature program (initially 80°C held for 2 min, then raised at 15°C min⁻¹ to 320°C, and finally held for 15 min) as described by Roy (2019a). The areas of each peak were converted into quantities of n-alkanes based on GC peak area of internal standard heneicosane (n-C₂₁ at 100 ng µl⁻¹).

Analysis of FFAs

The remaining half of the first portion of each crude extract of sesame leaves (Savitri and Nirmala) was mixed with diethyl ether and filtered through Whatman No. 41 filter paper. The extract was purified by TLC on silica gel G of 0.5-mm thickness by using n-butanol:acetic acid:water (4:1:5, v/v/v) as the mobile phase after discarding water. The band (R_f value of 0.69) was eluted from the silica gel layer with diethyl ether to get purified FFAs. Then, the purified FFAs were esterified with 3 ml BF₃-methanol followed by warming for 5 min in a hot water bath at 50–60°C and cooled. Hexane (40 ml) was added to this mixture followed by washing with saturated NaCl twice in a separating funnel. The aqueous layer of each sample was discarded and the hexane fraction was passed through 40 g anhydrous Na₂SO₄.

One portion of each esterified sample was used for GC-MS and another for GC-FID. The extraction of FFAs from each crude extract was separately repeated thrice followed by esterification as described by Roy (2019a). The extracts were analyzed with a Shimadzu GCMS-QP5050A to produce electron ionization (EI) mass spectra using HP-5MS column for GCMS-EI analysis by using a specified oven temperature program (initially held at 120°C for 2 min, then raised at the rate of 10°C min⁻¹ to 220°C, and finally held at 220°C for 15 min) as described by Roy (2019a). The areas of each peak were converted into quantities of FFAs based on GC peak area of internal standard methyl heneicosanoate (C_{21:0} at 100 ng µl⁻¹).

Bioassays

Wax chemicals for bioassays

Both natural n-alkanes and FFAs isolated from leaf surface wax of the two cultivars of sesame (Savitri and Nirmala) were

prepared in leaf equivalent (µg leaf⁻¹ ml⁻¹) amount dissolving in petroleum ether for different bioassays (olfactory attraction, oviposition, and feeding) of selected generalists (*S. obliqua*, *H. armigera*, and *S. litura*) through different treatments under defined conditions. Petroleum ether was used as the control solvent because both adults and larvae of the generalists were neither attracted nor deterred by it in preliminary bioassays. The synthetic individual n-alkanes, FFAs, and their mixtures mimicking the natural leaf wax (µg leaf⁻¹ ml⁻¹) were prepared by the same procedure as in naturally isolated chemicals. The de-waxed leaves for the bioassays were prepared by using fresh leaves in n-hexane for 1 min as described in the wax extraction process (Roy 2019a).

Insects for bioassays

Newly emerged (1–2 days old) F₄ females of *S. obliqua*, *H. armigera*, and *S. litura* were provisioned with water and starved for 12 h prior to use in olfactory attraction, and only 10% sucrose solution was provided as food during oviposition bioassays in different treatments. The newly hatched 4th instar larvae of each species were provisioned with water through moist filter papers and starved for 24 h prior to use for feeding bioassay in different treatments like their adults. Only 4th instar larvae were used in the bioassay experiments because they were most active with a higher consumption rate (CR) among the instars. Larval bioassays were also conducted to confirm the preference performance of adults (females) for their future generations. Always healthy individuals (females and 4th instar larvae) were selected and used once throughout the bioassay experiments with three replications for each pest species.

Adult olfactory bioassays

The behavioral responses of adult females were investigated in a Y-tube olfactometer (20 cm (length) stem and arms, 8 cm (diameter), 60° Y angle) as described by Roy (2019a). The stem of the olfactometer was connected to a porous glass vial ((8.0 cm (diameter) × 20.0 cm (length)) in which test insects were released. Each arm of the olfactometer was connected to a glass micro kit adapter (4.0 cm (diameter) × 6.0 cm (length)) fitted into a glass vial (8.0 cm (diameter) × 8.0 cm (length)). The membrane pump producing an airflow of 450 ml min⁻¹ was first purified by passing through a charcoal filter and the flow of purified air was adjusted to 150 ml min⁻¹ which led into left and right glass vials through the micro kit adapters. All the connections between different parts of the setup consisted of silicon tubes. One milliliter of solvent bearing 1-g leaf equivalent (µg leaf⁻¹ ml⁻¹) amount of identified n-alkanes and FFAs were applied (individually or in the mixture to Whatman No. 41 filter paper pieces (4 cm²) or on the leaf) as volatile cues and another only with solvent (petroleum

ether) or de-waxed leaf as control and allowed to evaporate the solvent in open space (1 min) under laboratory condition. These filter papers or leaves in different treatments were introduced into the glass vials attached with the olfactometer. One adult female of each pest (*S. obliqua*, *H. armigera*, and *S. litura*) was introduced into the porous glass vial attached with the olfactometer to measure the attractiveness as described by Roy (2019a).

The behavior of each female was observed for 3 min in the Y-tube because increasing the experimental time did not increase the number of responding insects as in Roy (2019a) and Mobarak et al. (2020a). A decision line was located on each side of the Y-tube and an individual crossing the line within 3 min from release with at least half the body was counted as a response. If no line was crossed after the experimental time had run out, the experiment was treated as no response (NR). To eliminate traces from previous trials, the tube was cleaned with petroleum ether followed by acetone and dried before a new individual was tested (Roy and Barik 2012b, 2014; Roy 2019a). Each experiment with one volatile sample was conducted until a total of 72 (24 × 3) females had used and after testing 12 insects, the olfactometer setup and the position of the two arms were systematically changed (rotated 180°) in order to avoid any positional biases.

Bioassays for cultivar preference by leaf cuticular wax chemicals

The dual-choice tests were performed for olfactory attraction of *S. obliqua*, *H. armigera*, and *S. litura* females to natural alkanes, FFAs, and wax in leaf equivalent ($\mu\text{g leaf}^{-1}$) amount along with intact leaf and mechanically damaged (50% lost) leaf of selected two sesame cultivars (Savitri and Nirmala). These bioassays were conducted to find the most preferred cultivar for them with three replications in different treatments under defined conditions as follows:

Condition 1: Alkane-treated filter paper vs. solvent having 1 treatment with natural n-alkanes present in cultivars Savitri and Nirmala.

Condition 2: FFA-treated filter paper vs. solvent having 1 treatment with natural FFAs present in cultivars Savitri and Nirmala.

Condition 3: Total wax chemical-treated filter paper vs. solvent having 1 treatment with natural wax present in cultivars Savitri and Nirmala.

Condition 4: Normal vs. de-waxed leaf with 2 treatments such as intact leaf, mechanically damaged (50% lost) leaf of cultivars Savitri and Nirmala.

The adult attraction index (AAI, in %) was determined for the 5 treatments under 4 conditions for each pest using the formula $[(T - C)/(T + C)] \times 100$, where T is the number of

adults (females) attracted in various treatments (normal leaf or filter paper) and C is the number of adults (females) attracted in controls (de-waxed leaf or solvent) with few modifications based on Singh et al. (2011).

Bioassays for individual synthetic wax chemicals

The same dual-choice tests for the olfactory attraction were conducted for the most common and abundant 10 n-alkanes and 11 FFAs identified from the most preferred cultivar of sesame (Savitri) individually as their synthetic analogs in leaf equivalent ($\mu\text{g leaf}^{-1}$) amounts to find the most preferred cues having minimum $\geq 50\%$ attractiveness with three replications in different treatments under defined conditions as follows:

Condition 1: Synthetic n-alkane-treated filter paper vs. solvent with 10 n-alkanes present in cultivar Savitri.

Condition 2: Synthetic FFA-treated filter paper vs. solvent with 11 FFAs present in cultivar Savitri.

The AAI (%) was determined for the 21 (10 n-alkanes + 11 FFAs) treatments under 2 conditions for each pest as in cultivar preference experiments.

Bioassays for most effective (synthetic and natural) wax chemicals

Similarly, dual-choice tests for olfactory attraction of *S. obliqua*, *H. armigera*, and *S. litura* females to the most preferred cues of selected synthetic n-alkanes (n-C₁₆, n-C₂₂, n-C₂₄, n-C₂₆) and FFAs (C_{12:0}, C_{14:0}, C_{18:1}) were used in the mixture as well as in combination (4 n-alkanes + 3 FFAs) for all the bioassay experiments because they were also produced more attractiveness than their individual cues for the pests. Similar experiments were performed with natural n-alkanes, FFAs, and their mixtures (n-alkanes + FFAs) along with mixtures of most preferred synthetic (4 n-alkanes, 3 FFAs, and 4 n-alkanes + 3 FFAs) mixtures in leaf equivalent ($\mu\text{g leaf}^{-1}$) amounts of cultivar Savitri. All the bioassays were conducted in the same manner as in synthetic individual compounds with three replications in different treatments under defined conditions as follows:

Condition 1: Alkane-treated filter paper vs. solvent with 2 treatments such as the mixture of the most preferred synthetic n-alkanes (n-C₁₆, n-C₂₂, n-C₂₄, n-C₂₆) and natural n-alkanes present in cultivar Savitri.

Condition 2: FFA-treated filter paper vs. solvent with 2 treatments such as the mixture of the most preferred synthetic FFAs (C_{12:0}, C_{14:0}, C_{18:1}) and natural FFAs present in cultivar Savitri.

Condition 3: Total wax chemical-treated filter paper vs. solvent with 2 treatments such as the mixture of synthetic

(4 n-alkanes + 3 FFAs) and natural (n-alkanes + FFAs) wax chemicals present in cultivar Savitri.

Condition 4: Normal vs. de-waxed leaf with 2 treatments such as intact leaf, mechanically damaged (50% lost) leaf of cultivar Savitri.

Condition 5: Combined synthetic mixture (4 n-alkanes + 3 FFAs)-treated normal vs. de-waxed leaf with 4 treatments such as one, two, three, and four leaf equivalent amount of the combined synthetic mixtures (4 n-alkanes + 3 FFAs) of cultivar Savitri.

The AAI (%) was determined for the 12 treatments under 5 conditions for each pest as in cultivar preference experiments.

Adult oviposition bioassays

Oviposition preference was assessed by using newly emerged 24 pairs of male and female of each pest species (*S. obliqua*, *H. armigera*, and *S. litura*) in a group with 3 groups for each ($24 \times 3 = 72$ pairs) in glass chambers ($40 \times 40 \times 40$ cm³) by using the natural and synthetic mixtures as in adult olfactory bioassays. The dual-choice test was conducted for each treatment in the said glass chambers covered with nylon net and the data were collected after 24-h intervals up to 96 h.

For the choice experiments, each leaf or filter paper was marked to create two halves vertically. One half was treated with the test compound and the other half was kept as a control. Each mixture was applied with a micropipette in leaf equivalent ($\mu\text{g leaf}^{-1}$) amount present in cultivar Savitri, and after evaporating the solvent, one pair of newly emerged moths was released in each glass chamber. Each chamber was provided with 10% sucrose solution as food and then kept in a BOD incubator as in mass culture. The leaf or filter paper of the three replicates having egg masses was detached from the glass chamber and eggs deposited on the surfaces were counted at the black head stage for 12 treatments under 5 conditions for each pest as in adult olfactory attraction bioassays (Table 6).

The oviposition preference index (OPI %) was determined for the 12 treatments under 5 conditions using the formula: $\text{OPI (\%)} = [(I - D)/(I + D)] \times 100$, where I is the number of eggs laid in various treatments (normal leaf or filter paper) and D is the number of eggs laid in controls (de-waxed leaf or solvent) as in adult olfactory attraction tests (Singh et al. 2011).

Larval olfactory bioassays

The attractiveness of 4th instar larvae of *S. obliqua*, *H. armigera*, and *S. litura* was conducted to investigate the possible pairing between respective larval attraction and adult oviposition preference in the same manner like adult olfactory bioassays in a miniature form of Y-tube olfactometer (12 cm

(length) stem and arms, 4 cm (diameter), 60° Y angle according to their body size) as described by Roy (2019a). The dual-choice tests were conducted by using the same natural and synthetic mixtures as in adult olfactory bioassays through 12 different treatments under 5 defined conditions. For each treatment, 72 larvae were tested with three replications for the selected pest species ($n = 24 \times 3$). The behavior of each larva was observed for 3 min in the Y-tube and larval attraction index (LAI, in %) was also calculated as in adult (AAI%) olfactory bioassays.

Larval feeding bioassays

Larval feeding bioassays of *S. obliqua*, *H. armigera*, and *S. litura* were conducted to trace the possible relationship between their larval attraction and feeding preference. Freshly collected mature leaves of the sesame cultivar (Savitri) were tested in 12 different treatments under normal vs. de-waxed leaf conditions. The solvent on the de-waxed leaf was dried at ambient temperature ($27 \pm 1^\circ\text{C}$) before the larvae were released onto the leaves. Healthy 4th instar larvae were selected for the experiment and placed separately in Petri dishes (9 cm in diameter) with normal and de-waxed leaves for each treatment. Each treatment was replicated three times for a pest and conducted with 72 (24×3) larvae per replication having 24 larvae in each group for each pest species. Leaf desiccation was prevented by using wet filter paper in each Petri dish.

Larvae were allowed to feed for 24 h and the area (cm²) consumed was measured for the 12 different treatments under 5 conditions as in adult olfactory bioassays where all treatments were with normal and de-waxed leaf other than filter paper and solvent (Table 8). Feeding index (FI %) was calculated for the 12 treatments under 5 conditions using the formula: $\text{FI (\%)} = [(F - N)/(F + N)] \times 100$, where F and N are the average consumption rate in normal and de-waxed leaf, respectively, as in LAI (%) calculation (Singh et al. 2011).

Data analysis

The data on total amounts of n-alkanes and FFAs of two cultivars (Savitri and Nirmala) of sesame were analyzed by one-way ANOVA followed by Tukey's HSD test. The data obtained on responses of *S. obliqua*, *H. armigera*, and *S. litura* bioassays to surface wax chemicals and their synthetic individuals as well as their mixtures were analyzed by the chi-square (χ^2) test based on the null hypothesis whether the ratio of individual choosing the stimulus vs. the control differs significantly from 1:1 (Zar 1999). Insects that did not respond (NR) to any one of the treatments were also included in the analyses. All the statistical analysis was conducted by using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

Surface wax

A single mature leaf of cultivars Savitri (1.6 ± 0.11 g) and Nirmala (1.4 ± 0.08 g) yielded 691.7 ± 22.12 and 531.0 ± 24.47 μg (mean \pm SE, $n = 3$) of surface wax, respectively. Out of the extracted waxes from a single leaf of Savitri and Nirmala represented 290.2 ± 3.97 and 249.9 ± 6.74 μg n-alkanes and 244.5 ± 13.15 and 190.9 ± 19.02 μg of FFAs, respectively, with the balance consisting of unidentified surface wax compounds (SM-Table 1). Total n-alkanes were significantly different ($F_{1,4} = 26.610$, $p = 0.007$) in the sesame cultivars (Savitri > Nirmala), whereas no significant differences were found in total surface waxes ($F_{1,4} = 5.794$, $p = 0.074$) and FFAs ($F_{1,4} = 3.198$, $p = 0.148$) at their respective leaf equivalent ($\mu\text{g leaf}^{-1}$) amounts (SM - Table 1).

Alkanes in leaf surface wax

Total 14 different n-alkanes were identified between n-C₉ and n-C₄₄ and, out of them, 13 and 11 types of n-alkanes were detected from the leaves of Savitri and Nirmala cultivars, respectively (Table 1). Hexacosane (n-C₂₆) and tetracosane (n-C₂₄) were predominant in Savitri (94.3 ± 7.27 $\mu\text{g leaf}^{-1}$) and Nirmala (44.5 ± 3.62 $\mu\text{g leaf}^{-1}$), respectively (Table 1). Amounts of all the identified n-alkanes were differed significantly ($F_{1,4} \geq 14.705$, $p \leq 0.019$) within the selected cultivars (Savitri > Nirmala) of sesame (Table 1).

FFAs in leaf surface wax

Total 12 different FFAs were identified between C_{9:0} and C_{20:0} and, out of them, 11 and 12 types of FFAs were detected from the leaves of Savitri and Nirmala cultivars, respectively (Table 2). Among them, octadecenoic acid (C_{18:1}) and nonanoic acid (C_{9:0}) were predominant in Savitri (110.8 ± 10.07 $\mu\text{g leaf}^{-1}$) and Nirmala (48.5 ± 4.68 $\mu\text{g leaf}^{-1}$), respectively (Table 2). All the identified FFAs were differed significantly ($F_{1,4} \geq 11.166$, $p \leq 0.029$), except tetradecanoic acid (C_{14:0}) within the selected cultivars (Savitri > Nirmala) of sesame (Table 2).

Adult attractions

Cultivar preference The highest attraction (%) and adult attraction index (AAI%) value of 86.4 ± 1.24 and $72.8 \pm 2.48\%$, respectively, were found in *S. obliqua* towards the intact leaves of Savitri cultivar followed by *H. armigera* and *S. litura* due to higher wax (691.7 ± 22.12 $\mu\text{g leaf}^{-1}$) content (Table 3). The AAI (%) values towards the treatments were in the order of intact leaf (condition 4) > natural wax (condition 3) > mechanically damaged leaf (condition 4) > FFAs (condition 2) > alkanes (condition 1) for the generalists (*S. obliqua* > *H. armigera* > *S. litura*) (Table 3). Thus, among the treatments, intact leaves (condition 4) of the selected cultivars (Savitri > Nirmala) were acted as the most preferred cues for the generalists (*S. obliqua* > *H. armigera* > *S. litura*) over the other treatments (Table 3).

Selection of most effective synthetic wax chemicals The attraction (%) towards the treatments over controls was always

Table 2 Composition of free fatty acids (FFAs) ($\mu\text{g leaf}^{-1}$) in two selected cultivars (Savitri and Nirmala) of sesame (*Sesamum indicum*, Pedaliaceae) determined during their growing season in 2019–2020

Free fatty acids (FFAs) ($\mu\text{g leaf}^{-1}$)	Savitri	Nirmala	$F_{1,4}$	p
Nonanoic acid (C _{9:0})	--	48.5 ± 4.68	107.288	< 0.001
Dodecanoic acid (C _{12:0})	24.2 ± 2.19	42.9 ± 4.15	15.925	0.016
Tetradecanoic acid (C _{14:0})	17.9 ± 1.63^a	17.3 ± 1.67^a	0.062	0.816
Pentadecanoic acid (C _{15:0})	13.4 ± 1.22^A	7.9 ± 0.77^A	14.240	0.020
Hexadecenoic acid (C _{16:1})	9.8 ± 0.89	6.2 ± 0.59^{AB}	11.166	0.029
Hexadecanoic acid (C _{16:0})	38.5 ± 3.49	9.4 ± 0.91	64.880	< 0.001
Octadecatrienoic acid (C _{18:3})	13.9 ± 1.26^A	5.9 ± 0.58^B	32.788	0.005
Octadecadienoic acid (C _{18:2})	4.6 ± 0.42^B	20.9 ± 2.02	62.641	< 0.001
Octadecenoic acid (C _{18:1})	110.8 ± 10.07	24.9 ± 2.40	68.966	< 0.001
Octadecanoic acid (C _{18:0})	5.9 ± 0.54^B	3.5 ± 0.34^C	14.551	0.019
Nonadecanoic acid (C _{19:0})	5.7 ± 0.52^B	2.9 ± 0.29^{CD}	21.816	0.010
Eicosanoic acid (C _{20:0})	5.0 ± 0.46^B	1.2 ± 0.11^D	68.013	< 0.001
Total FFAs	244.5 ± 23.151^a	190.9 ± 19.02^a	3.198	0.148

Note: Within rows and columns means followed by the same letters (lowercase and uppercase, respectively) are not significantly different ($p \geq 0.05$) by Tukey's HSD test

Table 3 Behavioral responses (females olfactory attraction) of three generalist pests (*Spilosoma obliqua* Walker (Arctiidae), *Helicoverpa armigera* Hübner (Noctuidae), and *Spodoptera litura* Fabricious (Noctuidae)) (mean \pm SE, n = 3) to leaf surface wax chemicals (n-alkanes and free fatty acids (FFAs) in leaf equivalent amount ($\mu\text{g leaf}^{-1}$) of two cultivars (Savitri and Nirmala) of sesame (*Sesamum indicum*, Pedaliaceae) under specified bioassay conditions

Adult (female) attraction	Savitri				Nirmala					
	Attraction (%)	χ^2 (df = 1)	NR (%)	AAI (%)	Attraction (%)	χ^2 (df = 1)	NR (%)	AAI (%)	$F_{1,4}$	p
<i>S. obliqua</i>										
Alkane-treated filter paper vs. solvent										
Natural alkane mixture	67.0 \pm 1.43 ^a	8.042	3.3	34.0 \pm 2.86 ^b	65.1 \pm 1.36 ^a	3.894	34.7	30.3 \pm 2.72 ^b	0.902	0.396
Fatty acid (FAs)-treated filter paper vs. solvent										
Natural FFA mixture	75.5 \pm 1.17	18.083	2.7	51.0 \pm 2.34	69.2 \pm 0.77	5.788	33.0	38.4 \pm 1.54	20.267	0.011
Total wax-treated filter paper vs. solvent										
Natural wax	85.6 \pm 0.77	35.136	2.7	71.2 \pm 1.54	73.4 \pm 0.51	9.384	29.3	46.9 \pm 1.01	173.056	0.001
Normal vs. de-waxed leaf										
Intact leaf	86.4 \pm 1.24	37.685	1.0	72.8 \pm 2.48	77.9 \pm 0.43	14.163	26.7	55.9 \pm 0.85	41.526	0.003
Damaged leaf (50% lost)	82.9 \pm 0.83	30.267	2.0	65.7 \pm 1.65	65.7 \pm 2.53	3.177	43.3	31.3 \pm 5.05	41.883	0.003
<i>H. armigera</i>										
Alkane-treated filter paper vs. solvent										
Natural alkane mixture	66.4 \pm 0.97 ^a	4.403	3.3	32.8 \pm 1.93 ^b	64.1 \pm 0.59 ^a	5.463	31.3	28.2 \pm 1.18 ^b	4.211	0.109
Fatty acid (FAs)-treated filter paper vs. solvent										
Natural FFA mixture	71.7 \pm 1.16 ^a	12.953	3.7	43.4 \pm 2.31	67.4 \pm 1.32 ^a	5.070	31.0	34.8 \pm 2.64	6.107	0.069
Total wax-treated filter paper vs. solvent										
Natural wax	77.4 \pm 1.26 ^a	20.915	2.7	54.8 \pm 2.52	73.5 \pm 3.06 ^a	11.163	23.0	46.9 \pm 6.13	1.411	0.301
Normal vs. de-waxed leaf										
Intact leaf	80.6 \pm 0.29 ^a	26.294	1.7	61.2 \pm 0.59	75.5 \pm 1.86 ^a	14.236	17.7	50.8 \pm 3.72	7.505	0.052
Damaged leaf (50% lost)	75.4 \pm 0.76	17.785	3.0	50.7 \pm 1.52	61.7 \pm 1.45	1.532	45.0	23.4 \pm 2.91	69.562	0.001
<i>S. litura</i>										
Alkane-treated filter paper vs. solvent										
Natural alkane mixture	64.1 \pm 1.20 ^a	3.870	2.7	28.2 \pm 2.40 ^b	60.6 \pm 0.89 ^a	3.146	36.7	21.2 \pm 1.77 ^b	5.617	0.077
Fatty acid (FAs)-treated filter paper vs. solvent										
Natural FFA mixture	67.2 \pm 2.17 ^a	8.259	4.0	34.4 \pm 4.33 ^b	68.9 \pm 1.12 ^a	4.956	37.7	37.8 \pm 2.24 ^b	0.465	0.533
Total wax-treated filter paper vs. solvent										
Natural wax	72.9 \pm 1.43 ^a	14.742	2.0	45.8 \pm 2.85 ^b	74.6 \pm 1.23 ^a	10.219	30.0	49.1 \pm 2.45 ^b	0.791	0.424
Normal vs. de-waxed leaf										
Intact leaf	77.9 \pm 1.12 ^a	21.634	2.7	55.8 \pm 2.24	71.9 \pm 1.63 ^a	9.020	25.7	43.8 \pm 3.25	9.243	0.038
Damaged leaf (50% lost)	74.9 \pm 0.79	17.119	3.0	49.8 \pm 1.59	61.6 \pm 2.77	1.394	49.3	23.2 \pm 5.54	21.373	0.010

Note: Within rows means followed by same letters are not significantly different ($p \geq 0.05$) by Tukey's HSD test; NR, no response (female); AAI, adult attraction index (female)

significantly higher in most effective n-alkanes (n-C₁₆, n-C₂₂, n-C₂₄, n-C₂₆) and FFAs (C_{12:0}, C_{14:0}, C_{18:1}) for all three generalists (Table 4). Highest AAI (%) was observed towards hexadecane (n-C₁₆) and octadecenoic acid (C_{18:1}) as 48.2 \pm 1.39 and 52.4 \pm 1.24 %, respectively in *S. obliqua* (Table 4). Among the treatments, the most preferred wax chemicals were in the order of FFAs (C_{18:1} > C_{12:0} > C_{14:0}) > n-alkanes (n-C₁₆ > n-C₂₄ > n-C₂₆ > n-C₂₂) as most attractive cues for the three generalists (*S. obliqua* > *H. armigera* > *S. litura*) over the other treatments (Table 4). All the AAI (%) values of the

selected generalist pests were significantly ($F_{2,6} \geq 5.970$, $p \leq 0.037$) differed among them except least preferred individual cues (Table 4).

Comparison of effective synthetic and natural wax chemicals

The attraction (%) towards any treatments over controls was always significantly ($\chi^2 \geq 5.463$, $df = 1$, $P < 0.05$) higher except natural alkane mixture in *S. litura* (Table 5). All the AAI (%) values were significantly ($F_{2,6} \geq 6.977$, $p \leq 0.027$) differed within the generalist pests except highest attractive

Table 4 Females olfactory attraction of three generalist pests (*Spilosoma obliqua* Walker (Arctiidae), *Helicoverpa armigera* Hübner (Noctuidae), and *Spodoptera litura* Fabricius (Noctuidae)) (mean \pm SE, n = 3) to synthetic individual leaf surface wax chemicals (common

and abundant n-alkanes and free fatty acids (FFAs) in leaf equivalent amount ($\mu\text{g leaf}^{-1}$) of sesame (*Sesamum indicum*, Pedaliaceae) cultivar (Savitri) under specified bioassay conditions

	<i>S. obliqua</i> (AAI%)	χ^2 (df = 1)	<i>H. armigera</i> (AAI%)	χ^2 (df = 1)	<i>S. litura</i> (AAI%)	χ^2 (df = 1)	$F_{2,6}$	<i>p</i>
Alkane-treated filter paper vs. solvent								
Nonene (n-C ₉)	27.8 \pm 2.35	3.202	25.9 \pm 2.84	2.707	18.8 \pm 1.64	1.724	4.154	0.074
Pentadecane (n-C ₁₅)	36.3 \pm 1.49 ^a	7.783	35.1 \pm 2.31 ^{ab}	7.146	28.9 \pm 0.99 ^b	5.510	5.532	0.043
Hexadecane (n-C₁₆)	48.2 \pm 1.39^a	13.206	47.0 \pm 2.96^{ab}	12.403	39.2 \pm 0.85^b	9.826	6.216	0.034
Eicosane (n-C ₂₀)	41.1 \pm 2.14	6.761	39.5 \pm 3.81	6.083	29.9 \pm 1.38	4.217	5.193	0.049
Docosane (n-C₂₂)	43.2 \pm 1.86^a	8.398	41.8 \pm 3.49^a	7.682	30.7 \pm 0.75	4.708	8.714	0.017
Tetracosane (n-C₂₄)	44.1 \pm 1.37^a	11.644	42.9 \pm 2.62^{ab}	10.894	35.9 \pm 0.86^b	8.650	6.110	0.036
Hexacosane (n-C₂₆)	43.7 \pm 1.48^a	10.661	42.4 \pm 2.79^{ab}	9.921	35.1 \pm 0.93^b	7.734	5.970	0.037
Octacosane (n-C ₂₈)	31.6 \pm 1.78 ^a	5.196	28.8 \pm 3.09 ^a	4.174	17.3 \pm 1.24	1.630	12.010	0.008
Hexatriacontane (n-C ₃₆)	28.5 \pm 2.03 ^a	3.847	26.9 \pm 2.49 ^a	3.337	19.5 \pm 1.40	2.036	5.657	0.042
Tetratetracontane (n-C ₄₄)	20.9 \pm 2.56 ^a	1.802	18.9 \pm 2.49 ^a	1.412	12.8 \pm 1.84	0.802	3.344	0.106
Fatty acid (FAs)-treated filter paper vs. solvent								
Dodecanoic acid (C_{12:0})	49.1 \pm 1.36^a	13.942	47.9 \pm 2.95^{ab}	13.126	40.2 \pm 0.82^b	10.462	6.282	0.034
Tetradecanoic acid (C_{14:0})	43.2 \pm 1.41^a	10.962	41.9 \pm 2.62^{ab}	10.226	34.9 \pm 0.89^a	8.066	6.028	0.037
Pentadecanoic acid (C _{15:0})	29.1 \pm 1.78 ^a	4.497	26.7 \pm 2.62 ^a	3.683	16.1 \pm 1.90	1.495	10.144	0.012
Hexadecenoic acid (C _{16:1})	28.8 \pm 1.89 ^a	4.172	26.8 \pm 2.52 ^a	3.508	17.8 \pm 0.44	1.722	10.866	0.010
Hexadecanoic acid (C _{16:0})	28.5 \pm 2.21 ^a	3.847	26.9 \pm 2.49 ^a	3.337	16.9 \pm 0.75	1.469	9.795	0.013
Octadecatrienoic acid (C _{18:3})	29.8 \pm 1.78 ^a	4.502	28.3 \pm 3.45 ^a	4.035	16.2 \pm 1.39	1.428	10.755	0.010
Octadecadienoic acid (C _{18:2})	32.8 \pm 1.95 ^a	5.073	31.2 \pm 2.73 ^a	4.498	20.8 \pm 0.78	2.223	6.504	0.031
Octadecenoic acid (C_{18:1})	52.4 \pm 1.24^a	16.972	51.3 \pm 2.90^{ab}	16.106	43.7 \pm 0.72	13.114	9.273	0.015
Octadecanoic acid (C _{18:0})	28.8 \pm 1.89 ^a	4.172	27.3 \pm 2.35 ^a	3.655	17.8 \pm 1.54	1.746	3.199	0.113
Nonadecanoic acid (C _{19:0})	21.0 \pm 2.93 ^a	1.610	18.8 \pm 2.88 ^a	1.220	11.9 \pm 2.09	0.635	4.007	0.078
Eicosanoic acid (C _{20:0})	25.3 \pm 2.18 ^a	2.917	23.6 \pm 2.43 ^a	2.457	17.4 \pm 1.54	1.592	4.022	0.078

Note: Within rows means followed by same letters are not significantly different ($p \geq 0.05$) by Tukey's HSD test; AAI, adult attraction index (female); The most attractive wax chemicals are presented in boldface

intact leaves with two leaf equivalent amount of combined synthetic mixture ($F_{2,6} = 4.083$, $p = 0.076$) in condition 5 (Table 5). The AAI (%) values in different conditions were in the order of condition 5 > condition 3 > condition 4 > condition 2 > condition 1 for all the generalists (*S. obliqua* > *H. armigera* > *S. litura*) (Table 5).

Oviposition responses

The oviposition choice (%) towards any treatments over controls were always significantly ($\chi^2 \geq 3.855$, $df = 1$, $p < 0.05$) higher except natural alkane mixture (condition 1) to *S. litura* and damaged leaf to all three generalists (Table 6). The OPI (%) values were without significant ($F_{2,6} \leq 3.108$, $p \geq 0.118$) differences within the generalist pests with few exceptions in conditions 2 and 5 as the plant was a potent host for them (Table 6). The OPI (%) values in different conditions were in the order of condition 5 > condition 4 > condition 3 > condition 2 > condition 1 for the generalists (*H. armigera* > *S. litura* > *S. obliqua*) (Table 6).

Larval attraction

The attraction (%) towards any treatments over controls was always significantly ($\chi^2 \geq 6.051$, $df = 1$, $p < 0.05$) higher except natural alkane mixture in *H. armigera* and *S. litura* (Table 7). All the LAI (%) values were significantly ($F_{2,6} \geq 5.479$, $p \leq 0.044$) differed within the generalist pests with few exceptions in conditions 2, 4, and 5 (Table 7). In all the treatments, LAI (%) values for the generalist pests were in the order of *S. obliqua* > *H. armigera* > *S. litura* except condition 5 (*H. armigera* > *S. litura* > *S. obliqua* as in oviposition preference) (Table 7). The LAI (%) values in different conditions were in the same order as in AAI (%) (Table 7).

Larval feeding

The feeding choice (%) towards any treatments over controls was always without any significant ($\chi^2 \leq 0.601$, $df = 1$, $p > 0.05$) differences because all treatments with intact leaves were acted as the most potent food for all the neonates

Table 5 Behavioral responses (females olfactory attraction) of three generalist pests (*Spilosoma obliqua* Walker (Arctiidae), *Helicoverpa armigera* Hübner (Noctuidae), and *Spodoptera litura* Fabricious (Noctuidae)) (mean \pm SE, n = 3) to most effective leaf surface waxchemicals (n-alkanes and free fatty acids (FFAs) in leaf equivalent amount ($\mu\text{g leaf}^{-1}$) of sesame (*Sesamum indicum*, Pedaliaceae) cultivar (Savitri) in different treatments under specified bioassay conditions

Adult attractions [wax chemicals (μg)]	<i>S. obliqua</i> (AAI%)	χ^2 (df = 1)	<i>H. armigera</i> (AAI%)	χ^2 (df = 1)	<i>S. litura</i> (AAI%)	χ^2 (df = 1)	$F_{2,6}$	p
Alkane-treated filter paper vs. solvent								
Synthetic n-alkane mixture ($\text{C}_{16}+\text{C}_{22}+\text{C}_{24}+\text{C}_{26}$) [240.624 \pm 3.462 μg]	40.0 \pm 1.65 ^{aA}	11.238	34.3 \pm 1.65 ^{abA}	8.267	28.2 \pm 2.71 ^{bAC}	5.654	8.125	0.020
Natural alkane mixture [290.242 \pm 3.966 μg]	34.0 \pm 2.86 ^{aA}	8.042	28.2 \pm 1.18 ^{abA}	5.463	21.2 \pm 1.77 ^{bA}	3.146	9.797	0.013
Fatty acid (FAs)-treated filter paper vs. solvent								
Synthetic FA mixture ($\text{C}_{12:0}+\text{C}_{14:0}+\text{C}_{18:1}$) [152.939 \pm 3.746 μg]	63.2 \pm 1.64 ^B	28.271	50.5 \pm 1.91 ^{aB}	17.886	47.4 \pm 0.86 ^{aBD}	15.642	30.022	0.001
Natural FFA mixture [244.487 \pm 13.151 μg]	51.0 \pm 2.34	18.083	43.4 \pm 2.31 ^B	12.953	34.4 \pm 4.33 ^C	8.259	6.977	0.027
Total wax-treated filter paper vs. solvent								
Synthetic (4 n-alkanes + 3 FFAs) mixture [393.563 \pm 4.126 μg]	78.3 \pm 1.83 ^C	43.375	61.9 \pm 2.52 ^C	26.914	54.7 \pm 1.83 ^D	21.204	33.660	0.001
Natural (n-alkanes + FFAs) mixture [534.729 \pm 7.412 μg]	71.2 \pm 1.54 ^C	35.136	54.8 \pm 2.52 ^B	20.915	45.8 \pm 2.85 ^B	14.742	29.454	0.001
Normal vs. de-waxed leaf								
Intact leaf [691.710 \pm 22.119 μg]	72.8 \pm 2.48 ^C	37.685	61.2 \pm 0.59 ^{aC}	26.294	55.8 \pm 2.24 ^{aD}	21.634	19.601	0.002
Damaged leaf (50% lost) [345.855 \pm 12.137 μg]	65.7 \pm 1.65 ^B	30.267	50.7 \pm 1.52 ^{aB}	17.785	49.8 \pm 1.59 ^{aB}	17.119	31.666	0.001
Synthetic (4 n-alkanes + 3 FFAs) mixture-treated normal vs. de-waxed leaf								
Intact leaf with one leaf equivalent amount ($\mu\text{g/leaf}$) of mixture [1085.273 \pm 16.421 μg]	82.9 \pm 2.72 ^{aD}	48.161	76.4 \pm 0.89 ^{aD}	41.275	67.5 \pm 0.89 ^E	31.719	20.108	0.002
Intact leaf with two leaf equivalent amount ($\mu\text{g/2 leaf}$) of mixture [1776.983 \pm 14.314 μg]	87.9 \pm 3.68 ^{aD}	55.576	83.0 \pm 1.57 ^{aD}	48.739	78.3 \pm 0.99 ^{aE}	43.341	4.083	0.076
Intact leaf with three leaf equivalent amount ($\mu\text{g/3 leaf}$) of mixture [2468.693 \pm 11.825 μg]	81.1 \pm 1.84 ^{aD}	46.564	77.4 \pm 1.55 ^{aD}	42.323	66.8 \pm 0.88 ^E	31.419	25.204	0.001
Intact leaf with four leaf equivalent amount ($\mu\text{g/4 leaf}$) of mixture [3160.403 \pm 10.626 μg]	83.9 \pm 1.85 ^{aD}	49.866	79.3 \pm 0.89 ^{aD}	44.389	69.6 \pm 0.89 ^E	34.148	31.876	0.001

Note: Within rows and columns means followed by same letters (lowercase and uppercase, respectively) are not significantly different ($p \geq 0.05$) by Tukey's HSD test; AAI, adult attraction index (female)

(Table 8). The FI (%) values were significantly ($F_{2,6} \geq 6.021$, $p \leq 0.037$) differed within the generalist pests with few exceptions in conditions 1, 3, 4, and 5 (Table 8). The FI (%) values in different conditions were in the same order for the generalists as in AAI (%) (Table 8).

Discussion

The cuticular wax of the selected sesame cultivars (Savitri and Nirmala) provides synergism through different sensory cues in suitable oviposition site selection for the studied generalists (*S. obliqua* > *H. armigera* > *S. litura*) like another arctiid moth

D. casignetum (Roy et al. 2012b; Roy and Barik 2012b, 2014; Roy 2019a). Total 14 n-alkanes from n-C₉ to n-C₄₄ and 12 FFAs (8 saturated + 4 monounsaturated) from C_{9:0} to C_{20:0} were detected from leaf cuticular wax of both sesame cultivars as major components with significant variations in their respective quantity ($\mu\text{g leaf}^{-1}$), as previously reported to other plants (Sarkar et al. 2013a, 2013b; Mukherjee et al. 2014; Mitra et al. 2017; Das et al. 2019; Mobarak et al. 2020a). The most predominant n-alkane and FFA of the cultivars were n-C₂₆ and C_{18:1}, respectively present in the Savitri cultivar. In other instances, 5 major n-alkanes (n-C₂₇, n-C₂₉, n-C₃₁ n-C₃₃, and n-C₃₅) were detected from sesame leaves, and among them, n-C₂₉ and n-C₃₃ were most abundant (Kim et al.

Table 6 Behavioral responses (gravid females oviposition preference) of three generalist pests (*Spilosoma obliqua* Walker (Arctiidae), *Helicoverpa armigera* Hübner (Noctuidae), and *Spodoptera litura* Fabricius (Noctuidae)) (mean \pm SE, n = 3) to most effective leaf

surface wax chemicals (n-alkanes and free fatty acids (FFAs) in leaf equivalent amount ($\mu\text{g leaf}^{-1}$)) of sesame (*Sesamum indicum*, Pedaliaceae) cultivar (Savitri) in different treatments under specified bioassay conditions

Oviposition preference [wax chemicals (μg)]	<i>S. obliqua</i> (OPI%)	χ^2 (df = 1)	<i>H. armigera</i> (OPI%)	χ^2 (df = 1)	<i>S. litura</i> (OPI%)	χ^2 (df = 1)	$F_{2,6}$	p
Alkane-treated filter paper vs. solvent								
Synthetic n-alkane mixture (C ₁₆ +C ₂₂ +C ₂₄ +C ₂₆) [240.624 \pm 3.462 μg]	41.8 \pm 0.69 ^{AA}	7.709	50.8 \pm 3.32 ^A	8.260	47.5 \pm 3.47 ^{AA}	5.812	2.612	0.153
Natural alkane mixture [290.242 \pm 3.966 μg]	29.3 \pm 0.76 ^B	3.855	39.4 \pm 3.79 ^{AB}	4.641	38.3 \pm 3.83 ^a	3.503	3.108	0.118
Fatty acid (FAs)-treated filter paper vs. solvent								
Synthetic FA mixture (C _{12:0} +C _{14:0} +C _{18:1}) [152.939 \pm 3.746 μg]	52.9 \pm 0.59 ^a	13.093	60.6 \pm 2.83 ^C	12.599	53.7 \pm 3.18 ^{aaB}	8.401	2.924	0.130
Natural FFA mixture [244.487 \pm 13.151 μg]	41.8 \pm 0.69 ^A	7.158	50.8 \pm 3.32 ^{AA}	7.670	53.9 \pm 3.17 ^{aaB}	7.813	5.479	0.044
Total wax-treated filter paper vs. solvent								
Synthetic (4 n-alkanes + 3 FAs) mixture [393.563 \pm 4.126 μg]	61.1 \pm 0.52 ^a	17.600	67.7 \pm 2.42 ^{aC}	15.979	64.2 \pm 2.62 ^a	12.950	2.532	0.159
Natural (n-alkanes + FAs) mixture [534.729 \pm 7.412 μg]	49.9 \pm 0.63 ^{AA}	10.012	57.9 \pm 2.69 ^{AA}	9.883	56.7 \pm 3.03 ^{AB}	9.183	3.059	0.121
Normal vs. de-waxed leaf								
Intact leaf [691.710 \pm 22.119 μg]	72.4 \pm 0.39 ^{aC}	27.835	77.3 \pm 1.79 ^{aD}	23.735	73.2 \pm 2.07 ^a	20.168	2.786	0.139
Damaged leaf (50% lost) [345.855 \pm 12.137 μg]	35.3 \pm 0.73 ^B	2.463	44.8 \pm 3.58 ^{AB}	2.884	26.0 \pm 4.19	0.961	8.590	0.017
Synthetic (4 n-alkanes + 3 FAs) mixture-treated normal vs. de-waxed leaf								
Intact leaf with one leaf equivalent amount ($\mu\text{g/leaf}$) of mixture [1085.273 \pm 16.421 μg]	77.8 \pm 0.33 ^{CD}	34.968	81.9 \pm 1.47 ^{DE}	29.084	82.9 \pm 1.39 ^C	27.063	5.190	0.049
Intact leaf with two leaf equivalent amount ($\mu\text{g/2 leaf}$) of mixture [1776.983 \pm 14.314 μg]	82.7 \pm 0.26 ^D	43.471	85.9 \pm 1.16 ^E	35.422	86.7 \pm 1.10 ^C	31.760	5.143	0.050
Intact leaf with three leaf equivalent amount ($\mu\text{g/3 leaf}$) of mixture [2468.693 \pm 11.825 μg]	82.1 \pm 0.27 ^D	41.307	85.4 \pm 1.20 ^E	33.744	83.9 \pm 1.32 ^C	29.562	2.610	0.153
Intact leaf with four leaf equivalent amount ($\mu\text{g/4 leaf}$) of mixture [3160.403 \pm 10.626 μg]	85.1 \pm 0.23 ^D	44.566	87.9 \pm 1.01 ^E	35.974	86.7 \pm 1.10 ^C	31.760	2.624	0.152

Note: Within rows and columns means followed by same letters (lowercase and uppercase, respectively) are not significantly different ($p \geq 0.05$) by Tukey's HSD test; OPI, oviposition preference index (gravid female)

2007). Total 9 n-alkanes (n-C₂₄ to n-C₃₀, n-C₃₂ and n-C₃₃) and 13 FFAs (C_{12:0} to C_{20:0}) were detected in the mature leaf surface wax of sunflower (*Helianthus annuus* L. (Asteraceae), cultivar PAC-36), where n-C₂₉ and C_{18:2}, respectively, were the most predominant (Roy and Barik 2012b, 2014). Similarly, a total of 18 n-alkanes (n-C₁₆ to n-C₃₆) and 13 FFAs (C_{12:0} to C_{20:0}) were detected in the leaf surface wax of jute (*C. capsularis*; cultivar: Sonali (JRC-321), Malvaceae), and among them, n-C₂₉ and C_{18:1}, respectively, were the most abundant (Roy 2019a). Furthermore, a total of 18 n-alkanes (n-C₁₅ to n-C₃₆) and 14 FFAs (C_{12:0} to C_{22:0}) were detected in the leaf surface wax of grass pea (*Lathyrus sativus* L., Fabaceae), and among them, n-C₁₅ and C_{16:1},

respectively, were most predominant (Mitra et al. 2020). Even, 20 n-alkanes (n-C₁₅ to n-C₃₆) and 13 FFAs (C_{12:0} to C_{21:0}) were identified from green gram (*Vigna radiata* L., Fabaceae) leaves, and among them, n-C₂₅ and C_{16:1}, C_{21:0}, respectively, were most abundant (Mobarak et al. 2020a).

Generally, n-alkanes and FFAs of different host plants can act as a short-range attractant for their respective insect pests (Schoonhoven et al. 2005; Li and Ishikawa 2006; Karmakar et al. 2016; Mitra et al. 2019). The short-distance behavioral responses of different insects were evaluated through different olfactometers (V-shaped, multi-tube, six-arm, Y-tube, etc. (Turlings et al. 2004, Koschier et al. 2000, Roy 2019a, Mitra et al. 2020)). The present Y-tube olfactometer bioassays

Table 7 Behavioral responses of 4th instar larvae (olfactory attraction) of three generalist pests (*Spilosoma obliqua* Walker (Arctiidae), *Helicoverpa armigera* Hübner (Noctuidae), and *Spodoptera litura* Fabricius (Noctuidae)) (mean \pm SE, n = 3) to most effective leaf surface wax chemicals (n-alkanes and free fatty acids (FFAs) in leaf equivalent amount ($\mu\text{g leaf}^{-1}$)) of sesame (*Sesamum indicum*, Pedaliaceae) cultivar (Savitri) in different treatments under specified bioassay conditions

Larval attraction [wax chemicals (μg)]	<i>S. obliqua</i> (LAI%)	χ^2 (df = 1)	<i>H. armigera</i> (LAI%)	χ^2 (df = 1)	<i>S. litura</i> (LAI%)	χ^2 (df = 1)	$F_{2,6}$	<i>p</i>
Alkane-treated filter paper vs. solvent								
Synthetic n-alkane mixture (C ₁₆ +C ₂₂ +C ₂₄ +C ₂₆) [240.624 \pm 3.462 μg]	38.7 \pm 0.96 ^{aA}	8.659	35.4 \pm 1.44 ^a	6.639	25.1 \pm 2.55 ^A	3.870	15.876	0.004
Natural alkane mixture [290.242 \pm 3.966 μg]	32.9 \pm 1.11 ^A	6.051	24.9 \pm 1.69	3.196	19.9 \pm 2.36 ^A	2.170	13.154	0.006
Fatty acid (FAs)-treated filter paper vs. solvent								
Synthetic FA mixture (C _{12:0} +C _{14:0} +C _{18:1}) [152.939 \pm 3.746 μg]	63.7 \pm 1.04 ^B	23.771	54.4 \pm 1.34 ^a	15.623	54.3 \pm 3.98 ^{aBC}	16.180	4.613	0.061
Natural FFA mixture [244.487 \pm 13.151 μg]	53.7 \pm 0.95 ^a	15.750	48.7 \pm 1.39 ^a	12.029	36.0 \pm 2.69	6.777	24.776	0.001
Total wax-treated filter paper vs. solvent								
Synthetic (4 n-alkanes + 3 FAs) mixture [393.563 \pm 4.126 μg]	72.8 \pm 1.67 ^{aC}	32.563	68.1 \pm 0.42 ^{aAB}	25.198	60.6 \pm 3.63 ^{BD}	20.231	7.054	0.027
Natural (n-alkanes + FAs) mixture [534.729 \pm 7.412 μg]	68.5 \pm 1.82 ^{aB}	27.912	62.1 \pm 1.40 ^{aAB}	20.283	49.7 \pm 3.17 ^C	13.617	17.868	0.003
Normal vs. de-waxed leaf								
Intact leaf [691.710 \pm 22.119 μg]	70.9 \pm 0.48 ^{aC}	30.038	70.7 \pm 0.48 ^{aB}	26.162	63.4 \pm 2.71 ^{aD}	21.775	7.058	0.027
Damaged leaf (50% lost) [345.855 \pm 12.137 μg]	66.1 \pm 1.39 ^{aC}	25.803	60.6 \pm 1.44 ^{abA}	18.583	57.1 \pm 3.35 ^{bc}	17.534	4.052	0.077
Synthetic (4 n-alkanes + 3 FAs) mixture-treated normal vs. de-waxed leaf								
Intact leaf with one leaf equivalent amount ($\mu\text{g/leaf}$) of mixture [1085.273 \pm 16.421 μg]	88.6 \pm 1.11 ^D	45.776	90.1 \pm 0.98 ^C	43.210	87.4 \pm 0.98 ^E	40.188	1.721	0.257
Intact leaf with two leaf equivalent amount ($\mu\text{g/2 leaf}$) of mixture [1776.983 \pm 14.314 μg]	93.4 \pm 1.05 ^D	52.369	96.3 \pm 0.09 ^C	50.740	95.3 \pm 1.08 ^E	50.474	5.479	0.044
Intact leaf with three leaf equivalent amount ($\mu\text{g/3 leaf}$) of mixture [2468.693 \pm 11.825 μg]	90.8 \pm 0.91 ^D	47.502	95.0 \pm 1.09 ^C	47.814	92.4 \pm 0.18 ^E	44.639	6.732	0.029
Intact leaf with four leaf equivalent amount ($\mu\text{g/4 leaf}$) of mixture [3160.403 \pm 10.626 μg]	93.1 \pm 0.21 ^D	50.276	93.7 \pm 1.34 ^C	46.894	93.8 \pm 2.28 ^E	46.599	0.068	0.935

Note: Within rows and columns means followed by same letters (lowercase and uppercase, respectively) are not significantly different ($p \geq 0.05$) by Tukey's HSD test; LAI, larval attraction index (4th instar larva)

revealed clear responses by the generalist pests (females and their larvae) to n-alkanes and FFAs present in leaf cuticular waxes of selected sesame cultivars. After reaching within a close range to the host plant, n-alkanes and FFAs were acted as a short-range attractant which facilitated oviposition induction in all gravid females as well as feeding in their larvae. Even, the role of olfaction was well documented in moths due to their typical nocturnal lifestyle (Cunningham et al. 1999). Visual (Goyret et al. 2007, Barragán-Fonseca et al. 2020), olfactory (Roy and Barik 2012b, 2014; Lucas-Barbosa et al. 2016; Das et al. 2019), tactile (Foster and Howard 1998; Roy 2019a), and gustatory (Feng et al. 2017) cues can themselves or in combinations with each other enhanced behaviors in host

selection for oviposition as well as for larval feeding (Carlsson et al. 1999; Bandoly et al. 2015).

The intact leaves of selected cultivars (Savitri > Nirmala) were acted as the most preferred olfactory cues for the three generalists (*S. obliqua* > *H. armigera* > *S. litura*) over the other treatments. At the individual level, the most preferred wax chemicals were in the order of FFAs (C_{18:1} > C_{12:0} > C_{14:0}) > n-alkanes (n-C₁₆ > n-C₂₄ > n-C₂₆ > n-C₂₂) as most attractive cues for the three generalists over the other treatments. The synthetic combination mixture mimicking the natural surface wax components having 4 n-alkanes (n-C₁₆, n-C₂₂, n-C₂₄, n-C₂₆) and 3 FFAs (C_{12:0}, C_{14:0}, C_{18:1}) was indicated significant olfactory attraction followed by oviposition

Table 8 Feeding preference of 4th instar larvae of three generalist pests (*Spilosoma obliqua* Walker (Arctiidae), *Helicoverpa armigera* Hübner (Noctuidae), and *Spodoptera litura* Fabricius (Noctuidae)) (mean \pm SE, $n = 3$) to most effective leaf surface wax chemicals (n-alkanes and free fatty acids (FFAs) in leaf equivalent amount ($\mu\text{g leaf}^{-1}$) of sesame (*Sesamum indicum*, Pedaliaceae) cultivar (Savitri) in different treatments under specified bioassay conditions

Larval feeding preference [wax chemicals (μg)]	<i>S. obliqua</i> (FI%)	χ^2 (df = 1)	<i>H. armigera</i> (FI%)	χ^2 (df = 1)	<i>S. litura</i> (FI%)	χ^2 (df = 1)	$F_{2,6}$	p
Alkane-treated normal vs de-waxed leaf								
Synthetic n-alkane mixture (C ₁₆ +C ₂₂ +C ₂₄ +C ₂₆) [932.334 \pm 9.414 μg]	29.6 \pm 1.71 ^{AB}	2.534	25.4 \pm 2.48 ^{AB}	1.642	24.9 \pm 0.83 ^{AC}	2.104	2.006	0.215
Natural alkane mixture [981.952 \pm 8.318 μg]	22.3 \pm 1.78 ^{abA}	1.446	17.1 \pm 2.58 ^{ba}	0.763	26.5 \pm 0.83 ^{aAC}	2.220	6.243	0.034
Fatty acid (FAs)-treated normal vs de-waxed leaf								
Synthetic FA mixture (C _{12:0} +C _{14:0} +C _{18:1}) [844.649 \pm 12.627 μg]	30.9 \pm 1.69 ^{aAB}	2.252	20.5 \pm 2.54 ^A	0.894	32.7 \pm 0.79 ^{aAB}	2.687	13.125	0.006
Natural FFA mixture [936.197 \pm 11.215 μg]	29.9 \pm 1.70 ^{aAB}	1.829	18.1 \pm 2.57 ^A	0.601	28.6 \pm 0.81 ^{aABC}	1.701	12.429	0.007
Total wax-treated normal vs de-waxed leaf								
Synthetic (4 n-alkanes + 3 FAs) mixture [1085.273 \pm 16.421 μg]	36.8 \pm 1.62 ^{BC}	2.615	29.8 \pm 2.41 ^B	1.591	35.5 \pm 0.78 ^B	2.902	4.570	0.062
Natural (n-alkanes + FAs) mixture [1226.439 \pm 14.943 μg]	36.8 \pm 1.62 ^{aBC}	2.180	23.5 \pm 2.50 ^{AB}	0.763	31.7 \pm 0.79 ^{aAB}	1.865	14.128	0.005
Normal vs. de-waxed leaf								
Intact leaf [691.710 \pm 22.119 μg]	33.9 \pm 1.66 ^B	2.848	24.3 \pm 2.49 ^{aAB}	1.308	20.9 \pm 0.85 ^{aC}	1.289	14.307	0.005
Damaged leaf (50% lost) [345.855 \pm 12.137 μg]	29.6 \pm 1.71 ^{AB}	2.534	25.4 \pm 2.48 ^{AB}	1.642	22.4 \pm 0.84 ^C	1.640	4.022	0.078
Synthetic (4 n-alkanes + 3 FAs) mixture-treated normal vs. de-waxed leaf								
Intact leaf with one leaf equivalent amount ($\mu\text{g/leaf}$) of mixture [1085.273 \pm 16.421 μg]	36.8 \pm 1.62 ^{BC}	2.615	29.8 \pm 2.41 ^B	1.591	35.5 \pm 0.78 ^B	2.902	4.570	0.062
Intact leaf with two leaf equivalent amount ($\mu\text{g/2 leaf}$) of mixture [1776.983 \pm 14.314 μg]	41.8 \pm 1.54 ^{aC}	2.437	33.7 \pm 2.34 ^{bBC}	1.432	35.5 \pm 0.78 ^{abB}	2.073	6.314	0.033
Intact leaf with three leaf equivalent amount ($\mu\text{g/3 leaf}$) of mixture [2468.693 \pm 11.825 μg]	46.0 \pm 1.47 ^{CD}	3.189	38.8 \pm 2.24 ^{aC}	2.051	39.6 \pm 0.75 ^{aD}	2.752	6.021	0.037
Intact leaf with four leaf equivalent amount ($\mu\text{g/4 leaf}$) of mixture [3160.403 \pm 10.626 μg]	52.9 \pm 1.34 ^D	3.618	40.4 \pm 2.21 ^{aC}	1.703	40.6 \pm 0.74 ^{aD}	2.344	21.399	0.002

Note: Within rows and columns means followed by same letters (lowercase and uppercase, respectively) are not significantly different ($p \geq 0.05$) by Tukey's HSD test; FI, feeding index (4th instar larva)

to all three generalists at leaf equivalent ($\mu\text{g leaf}^{-1}$) amount of cultivar Savitri. Thus, these pest species use visual (color and shape), olfactory (odorous n-alkanes and FFAs), tactile (surface intactness (ultra-structure)), and gustatory (cuticular wax) cues in a synergistic manner for oviposition (adults) and feeding (larvae) preference towards Savitri. The female choice was also supported by their neonates through probably tactile and gustatory responses for feeding on a normal leaf over the de-waxed leaf of Savitri like other insects (Roy et al. 2012a; Mitra et al. 2017; Das et al. 2019).

In other instances, two FFAs (C_{18:1}, C_{18:2}) act as host finding as well as an ovipositional cue for the navel orange worm,

Amyelois transitella (Walker) (Lepidoptera: Pyralidae) (Phelan et al. 1991). Five long-chain n-alkanes (n-C₂₆ to n-C₃₀) present in the epicuticular wax of corn (*Zea mays* L., Poaceae) and Japanese knotweed (*Fallopia japonica* (Houtt.) Ronse Decraene, Polygonaceae) leaves act as oviposition stimulants in the European corn borer *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) (Udayagiri and Mason 1997; Li and Ishikawa 2006). Even the arctiid moth *D. casignetum* were attracted by 5 predominant n-alkanes (n-C₁₈, n-C₂₃, n-C₂₄, n-C₂₈, and n-C₃₂) from leaf surface wax of sunflower at different concentrations (Roy and Barik 2012b). In addition, 6 FFAs (C_{16:0}, C_{16:1}, C_{18:0}, C_{18:1}, C_{18:2},

and C_{18:3}) from the same sunflower leaf surface wax produced the highest attraction to the same pest *D. casignetum* (Roy and Barik 2014). The synthetic combination mixture of 4 n-alkanes (n-C₁₇, n-C₁₈, n-C₂₇, n-C₂₉) and 5 FFAs (C_{16:0}, C_{16:1}, C_{18:1}, C_{18:2}, C_{18:3}) was most attractive to *D. casignetum* adults, whereas the same mixture excluding 2 n-alkanes (n-C₂₇, n-C₂₉) was also indicated oviposition preference at jute leaf at the equivalent amount (Roy 2019a). Even, 4 n-alkanes (n-C₂₅, n-C₂₇, n-C₂₉, n-C₃₆) out of 20 n-alkanes and 3 FFAs (C_{16:1}, C_{18:0}, C_{18:3}) out of 13 FFAs in combination were acted as a short-range attractant and oviposition stimulant in females of *S. obliqua* at green gram leaf equivalent amount (Mobarak et al. 2020a). Even, the obtained results indicated that the three generalists (*S. obliqua* > *H. armigera* > *S. litura*) were mostly attracted towards the synthetic blend of n-C₁₆, n-C₂₂, n-C₂₄, and n-C₂₆, and C_{12:0}, C_{14:0}, and C_{18:1} present in the leaf surface waxes of Savitri at leaf equivalent amount (μg leaf⁻¹). The degree of olfactory responses of the generalists (females > larvae) was maximum towards two leaf equivalent amount of combined synthetic (4 n-alkanes + 3 FFAs) mixture-treated intact leaf of Savitri due to optimum dose-response relationship. Similarly, oviposition and feeding preference of the generalists were maximum towards four leaf equivalent amount of the same synthetic mixture-treated intact leaf of Savitri due to a large amount of wax chemicals along with other physicochemical properties act in a synergistic manner. They also showed clear preference with significant differences towards normal leaf over the de-waxed leaf of the cultivars (Savitri > Nirmala).

The findings could explain the clue how gravid females of the generalist pests choose their oviposition site in such a perfect fashion for their potential hosts through different sensory modalities (visual (shape and color), olfactory (n-alkanes and FFAs as semiochemicals), tactile (leaf surface intactness (ultra-structure)), and gustatory (leaf surface wax)) in a synergistic manner for better survival and growth of their neonates like other insects (Carlsson et al. 1999; Mitra et al. 2019, 2020; Roy 2019a). Even, the behavioral responsiveness of the gravid females was also supported by their larval instars through attraction and feeding by these modalities also in a synergistic manner. Thus, the generalist females maximize their own fitness by laying eggs on their preferred host cultivar (Savitri > Nirmala) where their offspring perform best and which was supported by their larvae like other butterflies and moths (Birke and Aluja 2018; Mobarak et al. 2020a). Furthermore, this study also suggested that the synthetic blends of 4 n-alkanes and 3 FFAs along with the green leaf volatiles (GLVs (need to determine)) of the most preferred or susceptible cultivar of sesame (Savitri) can be used as a lure to develop baited trap. In addition, resistant cultivars like Nirmala can be used against the generalists for their ecological management in future.

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Declarations

Conflict of Interest The author declares no competing interests.

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