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FULL-LENGTH RESEARCH ARTICLE

Jute Leaf Physicochemical cue-mediated Behavioral Responses of *Diacrisia casignetum* Kollar

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Abstract The role of jute, *Chorchorus capsularis* (cv. Sonali; JRC-321), leaf physicochemical cues in the form of cuticular surface ultrastructures and wax chemicals mainly *n*-alkanes and free fatty acids (FFAs) on attraction and oviposition preference of *Diacrisia casignetum* Kollar (Lepidoptera: Arctiidae) was studied under laboratory conditions. The GC–MS and GC-FID analyses of mature jute leaf surface wax indicated the presence of 257.04 and 171.36 µg/leaf *n*-alkanes and FFAs, respectively. Eighteen *n*-alkanes from *n*-C_{16} to *n*-C_{36} and 13 FFAs from C12:0 to C20:0 were detected in the leaf surface wax. The cuticular surface ultrastructures and its chemicals have been demonstrated in this work to serve as cues for eliciting attraction and oviposition responses of the adults to mature jute leaves. The synthetic combination mixture mimicking the natural surface wax components of 4 *n*-alkanes (*n*-C17, *n*-C18, *n*-C27, *n*-C29) and 5 FFAs (C16:0, C16:1, C18:1, C18:2, C18:3) was most attractive to *D. casignetum* adults, whereas same mixtures excluding 2 *n*-alkanes (*n*-C27, *n*-C29) indicated significantly optimum oviposition preference at leaf equivalent (µg/leaf) concentrations that may be used for this pest management program as baited trap. The present study will also ensure sustainability of success in integrated pest management (IPM) in the form of green pest management (GPM) in the near future.

Keywords *Chorchorus capsularis* · Physicochemical cues · *n*-Alkanes · Free fatty acids · *Diacrisia casignetum*

Introduction

The expanding field of insect–plant interactions has long been focused on the process by which phytophagous insects find and accept their host plants [9, 20, 23, 38]. The interactions are primarily mediated by a set of physical and chemical cues specific to the plants [5, 17]. The sensory cues that elicit or inhibit attraction, oviposition and feeding clearly play crucial role in survival of most phytophagous insects, particularly in lepidopterans, because their neonates are often relatively immobile and thus depend on the judicious choice of host plant by the adult females [20, 36]. The host finding and oviposition site selection require a set of sensory modalities or cues (visual, olfactory, tactile and gustatory) of the females before laying eggs [20, 33]. After an insect flight on a plant, both physical and chemical cues of leaf surface become paramount in determining the suitability for attraction and oviposition [20, 39].

The leaf surfaces are covered with a thin layer of hydrophobic wax constituents, and it differs among plant species as well as within a plant during development [13, 24, 27, 29]. The leaf surface provides an enormous variety of microstructures, unicellular and multicellular outgrowths from the epidermis which provide an epic signature for host selection by insects [13, 29, 34]. The surface wax consists of alkanes, fatty acids, alkyl esters, alcohols, etc., and serves many physiological functions for protecting plants as well as in insect–plant interactions [13, 24, 27, 30–32, 38]. The importance of plant leaf

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alkanes and fatty acids as allelochemicals has been demonstrated in different insects in insect–plant interactions such as olfactory attractant [16, 18, 24, 27, 28, 30–32] and or oviposition attractant [15, 19].

The polyphagous pest, *Diacrisia casignetum* (Lepidoptera: Arctiidae), is one of the major limiting factors as defoliator (Fig. 1) in the successful cultivation of several economic crops including jute (*Chorchorus capsularis*), a natural fiber crop after cotton, in India and many other Asian countries [21, 22, 24–27]. Still in the era of modern agriculture, insects are controlled by chemical insecticides having detrimental effect to human health and environment [2, 7, 8].

So, it has become imperative now to have a precise knowledge on the physical and chemical cue-mediated interactions between the crop plant (jute) and the phytophagous insect (*D. casignetum*) for their ecologically sustainable green management. Hence, it is my considerable interest to find out the role of physical features of the mature jute leaf along with their surface wax chemicals (*n*-alkanes and FFAs) in behavioral responses (attraction and oviposition) of *D. casignetum* which may help in monitoring this pest as well as their green control strategy in the near future.

Materials and Methods

Jute Leaves Collection and Insect Mass Culture

Fresh mature (1–3 weeks old) jute leaves were randomly collected from the field near Chinsurah Rice Research Center (22°53′N, 88°23′E), Hooghly, West Bengal, India, for the experiment as in Roy [21, 22]. Insect mass culture was conducted at 27 ± 1 °C, 12 L:12 D with light intensity of 1500 lux, 65 ± 5% relative humidity (RH) in a biochemical oxygen demand (BOD) incubator, and from fourth generation onward, the bioassay was conducted for their behavioral responsiveness in the laboratory conditions as described by Roy and Barik [24–27].

Scanning Electron Microscopy (SEM) of Normal and De-waxed Leaves

Both abaxial and adaxial surfaces of a fresh and de-waxed mature leaves were separately mounted on aluminum holders (stabs) and coated with gold–palladium (2 nm thickness) to observe the ultrastructure of each surfaces of the leaf using Hitachi-made Scanning Electron Microscope (Model: S 530 with IB 2 ion cotter, Japan) as described by Adati and Matsuda [1] and Roy et al. [29].

Extraction, Identification and Quantification of Leaf Surface Wax

Freshly collected mature jute leaves of 200 g [fresh weight 612 ± 4 mg/leaf or 36 ± 0.53 cm^2/leaf (Mean ± SE)] were dipped in 2 L *n*-hexane for 1 min at room temperature for extraction of surface wax from the leaves which yielded a straw-colored extract without trace of chlorophyll [18, 29, 31, 32]. The crude extract was passed through Whatman No. 41 (Maidstone, UK) filter paper and was evaporated at room temperature (27 °C) to dryness. The

Fig. 1 Damage caused to the foliage of jute by the neonates of D. *casignetum*

extraction was repeated three times separately, and the dry extract yields were 216 ± 10 mg/200 g leaves. Each crude extract was then dissolved in 20 mL *n*-hexane and divided into four equal portions (equivalent to 50 g of leaves). The first and second ones were used for identification and quantification of *n*-alkanes and FFAs, and the remaining third and fourth ones after purification were used for attraction and oviposition bioassay, respectively. All solvents used were of analytical grade and purchased from E. Merck (Mumbai, India). All standard *n*-alkanes and fatty acids (FAs) (> 99% purity) were purchased from Sigma-Aldrich, Germany. Isolation, identification and quantification of *n*-alkanes and FFAs were done through TLC, IR spectroscopy, GC–MS and GC-FID in specific protocols as well as programming as in Sarkar et al. [31], Roy et al. [29], Sarkar et al. [32] and Roy and Barik [27], respectively.

Bioassay Experiments

Preparation of Surface Wax n-Alkanes and FFAs for Bioassay

Both natural *n*-alkanes and FFAs isolated from the mature jute leaf surface wax were prepared in leaf equivalent (µg/leaf/mL) amount dissolving in petroleum ether for different types of bioassay (attraction and oviposition) experiments. The petroleum ether was used as the control solvent because *D. casignetum* adults were neither attracted nor deterred by it in preliminary bioassay. To prepare the synthetic individual *n*-alkanes, FAs and their mixtures mimicking the natural leaf wax, the equivalent (µg/leaf/mL) amount was prepared by the same procedure as in naturally isolated chemicals.

Preparation of D. casignetum Adults for Bioassay

The F_4 onward generations of *D. casignetum* adults were collected from the mass culture for different bioassay experiments in the laboratory condition at 27 ± 1 °C, 60 ± 5% RH and light intensity of 1500 lux. Newly emerged females were provisioned with water and starved for 12 h prior to use in olfactory attraction, and only 10% sucrose solution provided as food during oviposition bioassays in different conditions. Females were used in bioassay because for oviposition they are guided by different physicochemical cues in suitable host finding for their future neonates. All the bioassay experiments were conducted with six replications.

Adult Attraction for *n*-Alkanes and FFAs

The effectiveness of *n*-alkanes and FFAs as olfactory attractants was evaluated in different conditions as they are low volatile substances that act as close range allelochemicals [14, 16, 24, 27, 28, 30]. The behavioral responses of adult females were investigated in a Y-tube olfactometer [20-cm-long (*l*) stem and arms, 8 cm diameter (*d*), 60° Y angle] (Fig. 2 in supplementary material) as described by Roy and Barik [24, 27]. Experiments with synthetic individual *n*-alkanes, FAs, mixtures of synthetic *n*-alkanes and FAs, mixtures of natural *n*-alkanes and FFAs and natural cuticular wax were conducted in the same manner as described by Roy and Barik [24, 27] and Sarkar et al. [31, 32].

Both natural *n*-alkanes and FFAs isolated from the mature jute leaf surface wax were prepared in leaf equivalent (µg/leaf/mL) amount dissolving in petroleum ether for the bioassay experiment. Adults were neither attracted nor deterred by petroleum ether in preliminary assays so it was used as the control solvent. To prepare the synthetic individual *n*-alkanes, FAs and their mixtures mimicking the natural leaf equivalent (µg/leaf/mL) amount were prepared by the same procedure as in naturally isolated chemicals. The stem of the olfactometer (Fig. 2 in supplementary material) was connected to a porous glass vial [8.0 cm (*d*) × 20.0 cm long (*l*)] in which test insects were released. Each arm of the olfactometer was connected to a glass-made micro-kit adapter fitted into a [4.0 cm (*d*) × 4.0 cm (*l*)] glass vial (Fig. 2 in supplementary material). The membrane pump producing an air flow of 450 mL/min was first purified by passing through charcoal filter, and the flow of purified air was adjusted to 150 mL/min 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 tubing. One mL of solvent bearing leaf equivalent (µg/leaf/mL) amount of identified natural *n*-alkanes and FFAs was applied separately to the Whatman No. 41 filter paper pieces (2 × 2 cm^2) used as volatile cues and another one with solvent (petroleum ether) used as control and allowed to evaporate the solvent in open space (1 min) under laboratory condition, and these filter papers were introduced into the glass vials attached with the olfactometer as described by Roy and Barik [24, 27]. The dual choice tests were conducted for both natural and synthetic *n*-alkanes and FAs individually and in mixtures. One adult female, *D. casignetum*, was introduced into the porous glass vial attached with the olfactometer to measure the attractiveness as described by Roy and Barik [24, 27]. 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. A decision line was located in 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 counted as "no response." To eliminate

traces from previous trials, the tube was cleaned with petroleum ether followed by acetone and dried before a new individual was tested. Each experiment with one volatile sample was conducted until a total of 72 (12 × 6) females had responded and after testing 6 insects the olfactometer setup and the position of the two arms were systematically changed (rotated 180°) in order to avoid positional bias. Experiments with synthetic individual *n*-alkanes, FAs, mixtures of synthetic *n*-alkanes and FAs, mixtures of natural *n*-alkanes and FFAs and natural cuticular wax were conducted in the same manner.

Adult Oviposition Bioassay

Oviposition preference was assessed by using newly emerged 12 pairs of male and female *D. casignetum* in a group with 6 replications (12 × 6=72 pairs) for the total of 20 different conditions in egg-laying chambers. The dual choice test with single jute leaf freshly excised from the plant, de-waxed leaf and or a similar leaf-shaped filter paper (36 cm^2) was conducted for different treatments accordingly in each glass jar (5 L) covered with nylon net, and the data were collected up to 96 h with 24-h interval. The leaves and or filter papers were watered during the observation period to prevent shrinkage. For the choice experiments with filter paper, each leaf-shaped filter paper (36 cm^2) was marked to create two halves vertically. One half was treated with the test compound in leaf equivalent (μg/leaf/mL) amount, and the other half of the paper was kept as a control treated with control solvent. Each compound was spread over the selected half filter paper in leaf equivalent (μg/leaf/mL) amount per test, and after evaporating the solvent, one pair of newly emerged adult moths (1:1 sex ratio) were released in each glass jar. Each jar was provided with 10% honey solution as food and then kept in a BOD incubator as in mass culture. The paper sheet of each replicate having egg masses was detached from the jar, and eggs deposited on treated and untreated surfaces were counted at the black head stage. The oviposition preference index (OPI%) was determined using the formula $[(T - C)/(T + C)] \times 100]$ where T is the number of eggs laid in various treatments or normal leaf and C is the number of eggs laid in controls [35]. The oviposition preference $[OA\% = T/(T + C) \times 100]$ and oviposition deterrence $[OD\% = C/(T + C) \times 100]$ were also calculated [35].

Data Analyses

The data on total amounts of *n*-alkanes and FFAs were analyzed by one-way ANOVA followed by Tukey HSD multiple-comparison test. The data obtained on responses of *D. casignetum* bioassay to surface waxes, individual compounds and combination of synthetic compounds were analyzed by Chi-square (χ^2) test based on the null hypothesis whether the ratio of individuals choosing the stimulus over the control solvent is differed significantly from the ratio of 1:1 [16, 18, 24, 27, 28, 30–32, 38, 40]. Individuals that did not respond to any one of the treatments in respective bioassay experiments were excluded from the analyses. All the statistical analysis was conducted by using SPSS software (SPSS 16.0; SPSS Inc., Chicago, IL, USA).

Results

Leaf Surface Ultrastructure

The waxy deposition on adaxial leaves surface revealed obscure cellular configurations in mature jute leaves. Ribs and furrows were distinctly visible. Both adaxial and abaxial surface showed undulations conspicuous with ridges of variable height and wide due to irregular waxy depositions (Fig. 1a, b). Undulated ridges and furrows reduced due to de-waxing of the leaf (Fig. 2c, d). The study on the relationship of the ultrastructure of both normal and de-waxed leaves with behavioral responses of the insect pest was conducted by oviposition preference test.

Surface Wax Composition

The *n*-hexane extracts of 100 g mature jute leaves yielded 108 ± 3 mg (mean ± SE) surface wax out of which *n*-alkanes and FFAs represented 42 ± 0.4 and 28 ± 0.1 mg, respectively, with the balance consisting of unidentified surface wax compounds. The identified *n*-alkanes of a single leaf represented 257.038 μg *n*-alkanes with the balance consisting of unidentified branched-chain alkanes (Table 1). Total 18 different *n*-alkanes were identified between *n*-C_{16} and *n*-C_{36} and expressed in leaf equivalent amount (μg/leaf) and in mol% (Table 1). Among them nonacosane (*n*-C_{29}) was the predominant (58.058 μg/leaf or 22.587 ± 0.109 mol%), whereas hexadecane (*n*-C_{16}) was detected in the lowest amount (1.301 μg/leaf or 0.506 ± 0.028 mol%). All the *n*-alkanes displayed significantly different patterns in the leaf surface waxes with few exceptions ($F_{17,51}$= 1699.32, $P < 0.0001$). The identified FFAs of a single leaf represented 171.359 μg FFAs with the balance consisting of unidentified fatty acids (Table 2). Thirteen (9 saturated and 4 mono-unsaturated) FFAs between C12:0 and C20:0 were detected in the jute leaf and expressed in leaf equivalent amount (μg/leaf) and in mol% (Table 2). Among them trioctadecanoin acid (C18:1) was the predominant one accounting 58.058 μg/leaf (6.122 ± 0.160 mol%), whereas least abundant (2.142 μg/

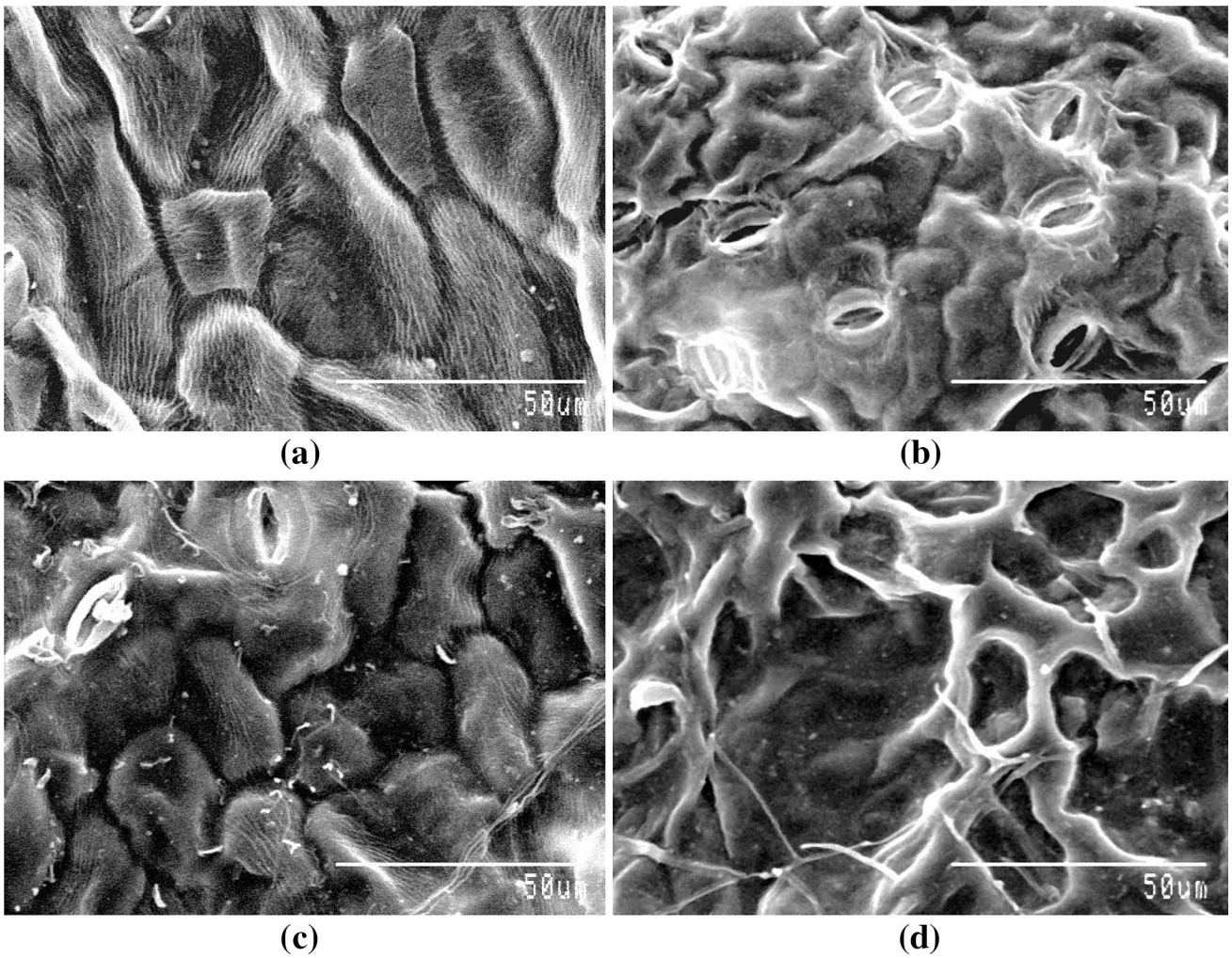

Fig. 2 Scanning electron micrographs of adaxial (**a**, **c**) and abaxial (**b**, **d**) surfaces of fresh (**a**, **b**) and de-waxed (**c**, **d**) mature leaves of jute, *C. capsularis*

leaf or 1.767 ± 0.038 mol%) was dodecanoic acid (C12:0). All the FFAs were significantly differed ($F_{12,36}$= 26,201.20, $P < 0.0001$) in the leaf surface wax with few exceptions.

Adult Attraction

A series of olfactometric tests (15 treatments) examining the effectiveness (minimum 60%) of synthetic *n*-alkanes and FAs individually, and their mixtures, as well as natural *n*-alkanes, FFAs and their mixtures in leaf equivalent amount (µg/leaf) on adult *D. casignetum*, are presented in Table 3. Among the natural chemicals, *n*-alkanes (257.04 µg/leaf), FFAs (171.36 µg/leaf) and surface wax (660.95 µg/leaf) attraction gradually increased (65.799 ± 2.672, 74.985 ± 4.436 and 75.197 ± 2.211%, respectively) in respective treatments. Within the identified chemicals, 4 *n*-alkanes [*n*-C17(a), *n*-C18(b), *n*-C27(c), *n*-C28(d)] and 5 FFAs [C16:0 (p), C16:1 (q), C18:1 (r), C18:2 (s), C18:3 (t)] individually, in mixture and in their combined mixture, elicited significantly positive responses to *D. casignetum*. Among the natural and synthetic chemicals, the highest positive attraction (81.893 ± 2.350%) was elicited by synthetic combined mixture (279.14 µg/leaf) of 4 *n*-alkanes and 5 FFAs (a + b+c + d+p + q+r + s+t) with highly significant differences (χ^2 = 25.825, *df* = 1, $P < 0.00001$), whereas the lowest attractiveness (63.225 ± 2.544%) was found in *n*-C17 (11.74 µg/leaf) with low significant differences (χ^2 = 2.906, *df* = 1, $P < 0.05$). All the responses significantly differed within the 15 treatments in both chemicals ($F_{14,84}$= 14.005, $P < 0.001$) and control solvent ($F_{14,84}$= 36.837, $P < 0.001$).

Oviposition Responses

The oviposition bioassay conducted by total of 20 treatments and having OA (%) > 50% was included, whereas

Table 1 Amount of *n*-alkanes (Mol% [mean ± SE] and leaf equivalent [μg/leaf (36 cm^2)]) in mature jute, *C. capsularis*, leaves

n-Alkanes	Carbon number	Retention time	Mol%	μg/leaf
Hexadecane	*n*-C16	6.417	0.506 ± 0.028	1.301
Heptadecane	*n*-C17	9.223	4.569 ± 0.031[a]	11.744[a]
Octadecane	*n*-C18	12.879	4.844 ± 0.024[a]	12.451[a]
Nonadecane	*n*-C19	12.975	0.632 ± 0.032	1.625
Icosane	*n*-C20	13.972	0.518 ± 0.029	1.331
Henicosane	*n*-C21	16.919	3.747 ± 0.012[b]	9.631[b]
Docosane	*n*-C22	17.015	0.551 ± 0.013	1.416
Tricosane	*n*-C23	21.006	2.415 ± 0.094[c]	6.207[c]
Tetracosane	*n*-C24	21.106	0.672 ± 0.032	1.727
Pentacosane	*n*-C25	23.078	3.088 ± 0.029[c]	7.938[c]
Hexacosane	*n*-C26	24.960	1.515 ± 0.034[d]	3.893[d]
Heptacosane	*n*-C27	27.060	22.424 ± 0.135[e]	57.639[e]
Octacosane	*n*-C28	28.717	1.036 ± 0.040	2.663
Nonacosane	*n*-C29	30.724	22.587 ± 0.109[e]	58.058[e]
Triacontane	*n*-C30	32.271	2.869 ± 0.007[c]	7.375[c]
Hentriacontane	*n*-C31	34.109	9.199 ± 0.029[g]	23.645[g]
Dotriacontane	*n*-C32	35.723	0.751 ± 0.030	1.931
Hexatriacontane	*n*-C36	45.087	18.076 ± 0.098[h]	46.463[h]

The same or no letter in superscript following the number indicates no significant difference ($P > 0.05$) in Tukey's HSD test within the column (Mol% and μg/leaf)

Table 2 Amount of FFAs (Mol% [mean ± SE] and leaf equivalent [μg/leaf (36 cm^2)]) in mature jute, *C. capsularis*, leaves

Free fatty acids (FFAs)	Carbon number	Retention time	Mol%	μg/leaf
Dodecanoic acid	C12:0	5.301	1.767 ± 0.038	2.142
Tridecanoic acid	C13:0	7.874	2.625 ± 0.027	2.420
Tetradecanoic acid	C14:0	9.697	3.232 ± 0.011[a]	5.079[a]
Pentadecanoic acid	C15:0	10.177	3.392 ± 0.006[b]	5.957[b]
Trihexadecenoin acid	C16:1	13.090	4.363 ± 0.004[c]	6.568[c]
Hexadecanoic acid	C16:0	14.533	4.844 ± 0.019[d]	17.319[d]
Heptadecanoic acid	C17:0	15.133	5.044 ± 0.1180[e]	3.655[e]
Tritetradecenoin acid	C18:3	17.056	5.685 ± 0.002[f]	14.360[f]
Trioctadecadienoin acid	C18:2	17.703	5.901 ± 0.078[g]	42.816[g]
Trioctadecenoin acid	C18:1	18.365	6.122 ± 0.160[h]	58.180[h]
Octadecanoic acid	C18:0	18.546	6.182 ± 0.034[i]	4.395[i]
Nonadecanoic acid	C19:0	21.687	7.229 ± 0.025[i]	4.190[i]
Eicosanoic acid	C20:0	27.865	9.288 ± 0.038[i]	4.278[i]

The same or no letter in superscript following the number indicates no significant difference ($P > 0.05$) in Tukey's HSD test within the column (Mol% and μg/leaf)

below levels were excluded from the experiment, respectively (Table 4). Within the treatments the highest OPI (46.999 ± 5.120%) was found in synthetic combined mixture of 2 *n*-alkanes and 5 FFAs (a + b+p + q+r + s+t) treated normal leaf (163.44 μg/leaf) having OA (%) of 73.499 ± 2.560%, while lowest OPI was found in *n*-C17 (5.288 ± 0.254%) having OA (%) of 52.644 ± 0.127%. All the treatments showed significant differences in all the three (OPI, OA and OD %) parameters ($F_{19,114}$= 30.908, $P < 0.001$) calculated from the oviposition responses. This type of responsiveness toward the normal leaf over the other treatments was due to the intact surface ultrastructure as well as applied synthetic principal surface wax components.

Table 3 Adult *D. casignetum* attraction % [mean ± SE] to natural and synthetic *n*-alkanes and FFAs at leaf equivalent amount (µg/leaf [36 cm^2]) in different conditions

Leaf equivalent chemicals	Amount (µg/mL/leaf)	Chemical attraction (%)	Control attraction (%)	Chi-square (χ^2)
Alkanes				
Natural alkanes	257.04	65.799 ± 2.672[a]	34.201 ± 2.672[a]	4.067 ± 1.156**
n-C17 (a)	11.74	63.225 ± 2.544[b]	36.775 ± 2.544[b]	2.906 ± 0.963*
n-C18 (b)	12.45	64.141 ± 2.525[b]	35.859 ± 2.525[b]	3.374 ± 1.034*
n-C27 (c)	57.64	65.704 ± 3.131[a]	34.296 ± 3.131[a]	3.443 ± 1.125**
n-C29 (d)	58.06	65.605 ± 3.787[a]	34.395 ± 3.787[a]	2.835 ± 1.082**
Synthetic mixture (a + b+c + d)	139.89	69.681 ± 2.109[c]	30.319 ± 2.109[c]	8.521 ± 1.508***
Free fatty acids (FFAs)				
Natural FFAs	171.36	74.985 ± 4.436[d]	25.015 ± 4.436[d]	7.368 ± 1.860****
C16:0 (p)	17.32	74.766 ± 2.398[d]	25.234 ± 2.398[d]	13.165 ± 1.956****
C16:1 (q)	6.57	75.951 ± 3.039[d]	24.049 ± 3.039[d]	11.736 ± 2.036****
C18:1 (r)	58.18	74.884 ± 3.878[d]	25.116 ± 3.878[d]	8.313 ± 1.883****
C18:2 (s)	42.82	75.387 ± 3.072[d]	24.613 ± 3.072[d]	10.980 ± 1.979****
C18:3 (t)	14.36	75.951 ± 3.039[d]	24.049 ± 3.039[d]	11.736 ± 2.036****
Synthetic mixture (p + q+r + s+t)	139.25	76.956 ± 5.016[d]	23.044 ± 5.016[d]	7.943 ± 2.022*****
Synthetic combined mixture (a + b+c + d+p + q+r + s+t)	279.14	81.893 ± 2.350[e]	18.107 ± 2.350[e]	25.825 ± 2.696*****
Natural leaf wax	660.95	75.197 ± 2.211[d]	24.803 ± 2.211[d]	14.893 ± 2.008***

The same letter in superscript following the number indicates no significant difference ($P > 0.05$) in Tukey's HSD test within the column (attraction %). The asterisk (*) following the Chi-square (χ^2) value indicates significant differences at $P < 0.05$, 0.01, 0.001, 0.0001 and 0.00001 by *, **, ***, **** and *****, respectively

Discussion

The host selection is a challenging task for mating, oviposition and feeding in lepidopterans. They require unique set of recognition cues for their complex behavioral process [10]. Complex stimuli have to be extracted from the environment and translated into a relevant behavioral output [33]. There are limitations in extraction of information from host volatiles due to noise of non-host volatiles in the ecological context. Specially, for a polyphagous pest, broader diet increases risk of oviposition on non-host or poor host along with evaluation time [3]. To resolve these questions, I have tested *D. casignetum* females in a series of behavioral assay for attraction and oviposition responses. There are a handful of studies that investigate antennal reaction to host plant volatiles [38] as well as olfactory cues with other stimuli [12]. Even though such findings are often discussed in other cases, unequivocal evidence for physicochemical cues used in navigation of lepidopterans has so far been scarce.

In this study, the result confirmed that *D. casignetum* females are able to use olfactory attraction in host plant selection for oviposition. In this study, the olfactometer tests demonstrated that the principal surface wax components as well as their synthetic analogs provide olfactory cues to attract adult female moths. In oviposition bioassay test in different conditions was also proved the use of physicochemical cues for their host or even oviposition site selection. Role of olfaction is well documented in moths due to their typical nocturnal lifestyle [6]. Even female moths can regulate navigation to make fine-tuned decisions for host selection and oviposition by visual [37, 39], olfactory [24, 27], tactile [11] and gustatory [4] cues separately or in combinations with each other.

The surface ultrastructure of the mature jute leaves represented epic pattern of wax deposition on both adaxial and abaxial sides like uniqueness of other plants [13, 24, 27, 29]. In the cuticular wax 18 types of *n*-alkanes from *n*-C_{16} to *n*-C_{36} and 13 types of FFAs from C12:0 to C20:0 were detected as major components with significant variations in their respective quantity (mol% and µg/leaf) as in other plants [16, 18, 24, 27, 29, 31, 32]. The highest olfactory attractiveness of *D. casignetum* females was found toward 4 *n*-alkanes (*n*-C17, *n*-C18, *n*-C27, *n*-C29) and 5 FFAs (C16:0, C16:1, C18:1, C18:2, C18:3) in combination mixture than individual compounds or their separate mixture or even natural wax components. The responsiveness helps the females in judicious host selection for oviposition. In oviposition bioassay the gravid females of *D. casignetum* were mostly preferred intact leaf rather

Table 4 Oviposition choice % [mean ± SE] of adult *D. casignetum* to the mature jute leaves, natural and synthetic *n*-alkanes and FFAs at leaf equivalent amount (μg/leaf [36 cm^2]) in different conditions

Oviposition conditions	Amount (μg/mL/leaf)	OPI (%)	OA (%)	OD (%)
Normal versus de-waxed leaf (36 cm^2)				
Intact leaf	–	39.125 ± 3.949^{a}	69.562 ± 1.974^{a}	30.438 ± 1.974^{a}
Pre infested leaf	–	22.859 ± 1.445^{b}	61.430 ± 0.722^{b}	38.570 ± 0.722^{b}
Mechanically damaged leaf	–	34.686 ± 3.260^{c}	67.343 ± 1.630^{c}	32.657 ± 1.630^{c}
Leaf disk (20 cm^2)	–	31.562 ± 2.810^{c}	65.781 ± 1.405^{c}	34.219 ± 1.405^{c}
n-Alkane treated filter paper (36 cm^2) versus solvent				
n-C17 (a)	11.74	5.288 ± 0.254^{d}	52.644 ± 0.127^{d}	47.356 ± 0.127^{d}
n-C18 (b)	12.45	5.840 ± 0.278^{d}	52.920 ± 0.139^{d}	47.080 ± 0.139^{d}
Synthetic *n*-alkanes mixture (a + b)	24.19	10.680 ± 0.495^{e}	55.340 ± 0.248^{e}	44.660 ± 0.248^{e}
Natural *n*-alkanes	257.04	11.487 ± 0.537^{e}	55.744 ± 0.268^{e}	44.256 ± 0.268^{e}
Free fatty acids (FFAs) treated filter paper (36 cm^2) versus solvent				
C16:0 (p)	17.32	6.140 ± 0.291^{d}	53.070 ± 0.145^{d}	46.930 ± 0.145^{d}
C16:1 (q)	6.57	9.835 ± 0.381^{e}	54.918 ± 0.190^{e}	45.082 ± 0.190^{e}
C18:1 (r)	58.18	13.009 ± 0.612^{e}	56.504 ± 0.311^{e}	43.496 ± 0.311^{e}
C18:2 (s)	42.82	18.930 ± 1.053^{f}	59.465 ± 0.527^{f}	40.535 ± 0.527^{f}
C18:3 (t)	14.36	14.604 ± 0.720^{e}	57.302 ± 0.360^{e}	42.698 ± 0.360^{e}
Synthetic FFAs mixture (p + q+r + s+t)	139.25	35.978 ± 3.379^{c}	67.989 ± 1.690^{c}	32.011 ± 1.690^{c}
Natural FFAs	171.36	19.449 ± 1.136^{f}	59.725 ± 0.568^{f}	40.275 ± 0.568^{f}
Chemicals treated filter paper (36 cm^2) versus solvent				
Synthetic *n*-alkanes + FFAs combination mixture on filter paper	163.44	28.908 ± 2.218^{g}	64.454 ± 1.109^{g}	35.546 ± 1.109^{g}
Natural wax on filter paper	660.95	25.325 ± 1.735^{bg}	62.663 ± 0.867^{bg}	37.337 ± 0.867^{bg}
Chemicals treated on normal versus de-waxed leaf (36 cm^2)				
Synthetic *n*-alkanes+ FFAs combination mixture	163.44	46.999 ± 5.120^{h}	73.499 ± 2.560^{h}	26.501 ± 2.560^{h}
Natural *n*-alkanes+ FFAs combination mixture	428.4	32.622 ± 2.736^{c}	66.311 ± 1.368^{c}	33.689 ± 1.368^{c}

The same letter in superscript following the number indicates no significant difference ($P > 0.05$) in Tukey's HSD test within the column (oviposition preference index [OPI], oviposition acceptance [OA] and oviposition deterrence [OD] %)

than the other but their degree of preference can be amplified when the intact leaf pretreated with the same combined synthetic mixture as in olfactory attraction excluding two *n*-alkanes (*n*-C27 and *n*-C29).The finding of both olfactory and oviposition bioassay in different conditions can explain the clue how *D. casignetum* females choose their oviposition site in such a perfect passion through host physicochemical cues for better survival and growth of their neonates. It is apparent from my study that understanding the potential semiochemicals in the behavioral responses of insects could play a significant role in any biointensive pest management strategy if standardized protocols for identification of the semiochemicals and their appropriate delivery systems for field applications are available.

In summary, the bioassay (olfactory attraction and oviposition) experiments proved that how host physicochemical cues were used by *D. casignetum* to sharpen their decision for attraction and ultimate oviposition site selection on their preferred host plants or rather plant parts. So, for management of the notorious pest, *D. casignetum*, on different economic crops like sunflower, safflower, green gram, castor, sesame, including jute, through adopting and mimicking the combination mixture of synthetic semiochemicals will be an alternative, ecofriendly and sustainable strategy over the traditional pest management strategies. The present study also ensures the application of the chemical combination mixture for further research on this notorious defoliator pest in integrated pest management (IPM) model in the form of green pest management (GPM) or rather ecological pest management (EPM) in the near future.

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Author's Contributions NR designed the whole study including sample collection, chemical analysis and data analysis and prepared the manuscript.

Compliance with Ethical Standards

Conflict of interest The author declares that there is no conflict of interest.

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