



# Behavioral pattern of generalist and specialist insect pests to brassicaceous leaf cuticular *n*-alkanes and free fatty acids

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Received: 2 March 2022 / Accepted: 22 August 2022 / Published online: 8 September 2022  
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## Abstract

The role of leaf cuticular wax of cabbage, *Brassica oleracea* L., rapeseed, *B. campestris* L., and Indian mustard, *B. juncea* L. in host preference by two generalists, *Spilarctia obliqua* Walker and *Pieris brassicae* L., and a specialist, *Plutella xylostella* L. was investigated under laboratory conditions. A total of 25 *n*-alkanes from *n*-C<sub>14</sub> to *n*-C<sub>36</sub> and 15 free fatty acids (FFAs) from C<sub>12:0</sub> to C<sub>22:0</sub> were detected from leaf cuticular wax of three crops with significant variations in their respective quantities (µg leaf<sup>-1</sup>). The most preferred wax chemicals were consisted of 5 *n*-alkanes (*n*-C<sub>16</sub>, *n*-C<sub>18</sub>, *n*-C<sub>20</sub>, *n*-C<sub>22</sub>, *n*-C<sub>30</sub>) and 5 FFAs (C<sub>12:0</sub>, C<sub>16:0</sub>, C<sub>18:3</sub>, C<sub>18:2</sub>, C<sub>18:1</sub>) for the pests. Highest attraction index (AI%) and oviposition preference index (OPI%) were in the order of *P. brassicae* > *P. xylostella* > *S. obliqua* and *P. xylostella* > *P. brassicae* > *S. obliqua*, respectively, toward the combined synthetic mixture (5 *n*-alkanes + 5 FFAs) treated leaf of cabbage due to higher amount of wax chemicals in the specific combination. Both generalist and specialist have same patterns of olfaction and oviposition preferences with different magnitude of responses. Thus, the synthetic blends of same 5 *n*-alkanes and 5 FFAs of the most preferred cabbage cultivar might be used as lure to develop baited trap as well as less preferred crop (rapeseed and Indian mustard) species might be used as main crop with cabbage as trap crop against the pests for their sustainable ecological management in near future.

**Keywords** *Brassica oleracea* · *B. campestris* · *B. juncea* · *Spilarctia obliqua* · *Pieris brassicae* · *Plutella xylostella* · *n*-alkanes · Free fatty acids · Baited trap · Trap crop

## Introduction

Brassicaceous vegetables like cabbage and oil seeds are most popular around the world in respect of its great potential for providing food security (Biswas et al. 2019; Gawariya et al. 2015; Neik et al. 2017; Obermeier et al. 2022; Tandayu et al. 2022). Cabbage, *Brassica oleracea* L. var. *capitata*, is one of the most important vegetables grown throughout the world (Koza et al. 2018). After China, India is the largest grower of cabbage in world (Salim and Abed 2015). Bearing leafy green heads, the cabbage is used as salad, vegetable, food (cooked/raw), as well as for medicinal purposes (Tripathi 1999; Koza et al. 2018). Rapeseed (*Brassica campestris* L.) and Indian mustard (*Brassica juncea* L. Czern & Coss.) are the major oilseed crops in the world after soybean and groundnut, respectively (Singh et al. 2010; Dutta 2016).

India is one of the largest grower, producer, importer, as well as exporter of these oilseed crops in the world (Barick et al. 2020). The oil content of the rapeseed and mustard ranges about 42% and 38–40%, respectively (Biswas et al. 2019). In southeast Asian countries a complex of more than 30 beneficial and harmful insect pests are found on these crops (Ali and Rizvi 2007; Golizadeh et al. 2009; Kumar et al. 2020; Song et al. 2022). However, genetic breeding and mixed cropping of these crops were conducted for development of improved cultivars with multiple insect pest resistances/tolerances for their better production (Hussain et al. 2020; Roy 2021a; Obermeier et al. 2022).

Among the insect pests, the hairy caterpillar, *Spilarctia obliqua* (Walker, 1855) (Lepidoptera: Arctiidae) (Gupta and Bhattacharya 2008; Warad and Kalleswaraswamy 2017), cabbage caterpillar, *Pieris brassicae* (Linnaeus, 1758) (Lepidoptera: Pieridae) (Ali and Rizvi 2007; Bhowmik and Gupta 2017), and the oligophagous, diamondback moth, *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) (Sarfranz et al. 2007; Karmakar et al. 2022) are cosmopolitan in distribution and most serious defoliator of different

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cruciferous crops. Their full-grown larvae cause severe defoliation and significant reduction in yield (Kumar et al. 2018; Gupta and Bhattacharya 2008). In many countries they have developed resistance to most synthetic insecticides used against them (Sarfranz and Keddie 2005; Hasan and Ansari 2010; Kumar and Ali 2010; Passos et al. 2020). In addition, the indiscriminate use of broad-spectrum insecticides are considered responsible for its high pest status (Aktar et al. 2009; Deguine et al. 2021). Consequently, a comprehensive behavioral study of these pests on most common brassicaceous crops (cabbage, *B. oleracea* L., cv. NS-183, rapeseed, *B. campestris* L. cv. TS 38, and Indian mustard, *B. juncea* L. Czern & Coss., cv. B 85) (Atri et al. 2012; Banerjee et al. 2018; Barick et al. 2020) is needed to promote integrated pest management (IPM) and to reduce the reliance on the chemical pesticides.

Herbivorous insects recognize their host plants by several physicochemical cues (Renwick and Chew 1994; Schoonhoven et al. 2005; Lucas-Barbosa et al. 2016). The first physical contact between an herbivorous insect and host plant occurs on the leaf cuticular surfaces which act as low volatile cues for host acceptance (Chapman and Bernays 1989; Derridj et al. 1996; Jetter and Schäffer 2001; Fernández et al. 2019; Song et al. 2022). Particularly, in lepidopterans it is crucial as their neonates are often relatively immobile and thus depend on the judicious choice of host plant by the adult females (Jetter et al. 2000; Müller and Riederer 2005). Even, the epicuticular waxes protect plant tissues from uncontrolled non-stomatal water loss, UV radiation, defense against pathogens, etc. (Wang et al. 2015). Epicuticular waxes mainly consist of long-chain alkanes, FFAs, esters, aldehydes, and alcohols, which composition varies widely within a species or cultivars of a species (Jetter et al. 2006; Mitra et al. 2020; Debnath et al. 2021). The importance of leaf alkanes and FFAs as semio-chemicals has been demonstrated for management of different insect species (Eigenbrode and Pillai 1998; Müller and Hilker 2001; Das et al. 2019; Little et al. 2019). Specially, low volatile *n*-alkanes and FFAs serve an important role in insect–plant interactions, like olfactory attractant (Roy et al. 2012a, b; Karmakar et al. 2016; Malik et al. 2017) and or oviposition stimulant (van Loon et al. 1992; Parr et al. 1998; Li and Ishikawa 2006). There are a handful of studies that investigated the attraction to host plant volatiles (Ikeura et al. 2010; Feng et al. 2017; Hussain et al. 2020; Song et al. 2022) but unequivocal evidence for host wax chemicals (*n*-alkanes and FFAs) used in olfaction and oviposition site selection by these insect pests has so far been scarce.

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 the three widely used crops (cabbage [cv. NS-183], rapeseed [cv.

TS 38], and Indian mustard [cv. B 85]), (ii) to find the role of respective leaf surface (epicuticular) wax chemicals followed by their synthetic analogs and their mixtures (*n*-alkanes and FFAs) in short-range attraction and oviposition of the three pests (*S. obliqua*, *P. brassicae*, and *P. xylostella*) through different bioassay experiments under laboratory conditions, (iii) to find the pattern of olfaction and oviposition preferences of the generalists (moth and butterfly) and specialist (moth) to the wax chemicals, and (iv) to find out the most effective combination of wax chemicals (*n*-alkanes and FFAs) in attraction and oviposition of the pests for designing a baited trap as well as more susceptible crops as trap crop for integrated pest management (IPM) in near future.

## Materials and methods

### Plants

Three widely used brassicaceous crops, like cabbage, *Brassica oleracea* L. (cv. NS-183), rapeseed, *B. campestris* L. (cv. TS 38), and Indian mustard, *B. juncea* L. (cv. B 85) (Banerjee et al. 2018; Biswas et al. 2019; Barick et al. 2020), were cultivated in a selected field in West Bengal, India (22° 53' N, 88° 23' E, 13-m above sea level) during 2020–2021 growing season. Nine plots [each plot 10 m × 10 m; soil organic matter 5.3 ± 0.2%, pH 7.7, average relative humidity (RH) 70 ± 15%, photoperiod 14 L:10 D at 30–36 °C] were prepared for cultivation of the crops with average plant density of 9 ± 3, 34 ± 4, and 40 ± 4 plants/m<sup>2</sup>, respectively. The selected crop species 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 cultivation of another crop and all plots were maintained without any insecticide spraying. Two to three mature leaves were collected from each 5–7-week-old plant at 6 AM. Three separate batches of around 100-g leaves of each crop (cabbage leaf [*n* = 7], rapeseed leaf [*n* = 51], and Indian mustard leaf [*n* = 55]) were collected from the respective plots for extraction of leaf surface waxes. Only mature leaf surface waxes of crops were considered during this study because the herbivores generally prefer to feed on these leaves.

### Insects

Three lepidopteran pests, polyphagous, *S. obliqua* Walker (Arctiidae), *P. brassicae* L. (Pieridae), and oligophagous, *P. xylostella* L. (Plutellidae) adults (males and females), were collected by light trap from the similarly cultivated sesame (*Sesamum indicum*, cv. Rama, Pedaliaceae) plants growing in the field in West Bengal, India and placed them on the same sesame leaves for egg laying. Newly emerged first instar larvae (F<sub>1</sub>) of each pest species were placed separately

on the same host leaves for feeding and they were kept at  $27 \pm 1$  °C,  $70 \pm 10\%$  RH, and 14 L:10 D photoperiod with light intensity of 1500 lx in a Biological Oxygen Demand (BOD) incubator as in Roy (2019b, 2020). Three generations ( $F_1$ – $F_3$ ) of each pest species were completed on the same host leaves. The  $F_4$  mature females (1–2 days old) of each species were used for olfactory and oviposition bioassays in laboratory conditions. The larvae and adults (females) of *S. obliqua*, *P. brassicae*, and *P. xylostella* were not reared on the selected host plants (cabbage, rapeseed, and Indian mustard) other than sesame based on Hopkins' host-selection principle (Barron 2001) to avoid any biasness to leaf surface waxes of respective crop species during their bioassays.

### Extraction of leaf surface wax

Freshly collected mature leaves of about 100 g for each crop (cabbage [cv. NS-183], rapeseed [cv. TS 38], and Indian mustard [cv. B 85]), respectively (ESM Table 1) with three replications were dipped in 2L *n*-hexane

separately for 1 min at room temperature for extraction of their surface wax which yielded straw-colored extracts without trace of any chlorophyll (Mitra et al. 2020). The crude extract was passed through Whatman No. 41 (Maidstone, UK) filter paper and was evaporated at room temperature (20–22 °C) to dryness. The extraction was repeated three times separately for each crop and the dry extract (wax) were yielded  $16.667 \pm 0.371$ ,  $38.400 \pm 0.519$ , and  $73.067 \pm 0.867$  mg  $100 \text{ g}^{-1}$  in cabbage, rapeseed, and Indian mustard leaves, respectively (ESM Table 1). Each crude extract was then dissolved in 30-ml *n*-hexane and divided into three equal portions (equivalent to 33.33 g of leaves), first one used for identification and quantification of *n*-alkanes and FFAs, whereas remaining second and third one after purification used for attraction and oviposition bioassays, 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).

**Table 1** Composition of *n*-alkanes ( $\mu\text{g leaf}^{-1}$ ) in plant surface waxes (Mean  $\pm$  SE,  $n=3$ ) of three selected brassicaceous crops (cabbage, *Brassica oleracea* L. [cv. NS-183], rapeseed, *B. campestris* L. [cv. TS

38], and Indian mustard, *B. juncea* L. [cv. B 85]) determined during their growing season in 2020–2021

Alkanes ( $\mu\text{g leaf}^{-1}$ )	Cabbage	Rapeseed	Indian mustard	$F_{2,6}$	<i>P</i>
<i>n</i> -Tetradecane ( <i>n</i> -C <sub>14</sub> )	4.261 $\pm$ 0.113 <sup>a</sup>	4.647 $\pm$ 0.047 <sup>b</sup>	1.347 $\pm$ 0.080 <sup>c</sup>	457.361	<0.001
<i>n</i> -Pentadecane ( <i>n</i> -C <sub>15</sub> )	39.944 $\pm$ 1.058 <sup>a</sup>	20.253 $\pm$ 0.204 <sup>b</sup>	12.164 $\pm$ 0.724 <sup>c</sup>	363.463	<0.001
<i>n</i> -Hexadecane ( <i>n</i> -C <sub>16</sub> )	105.645 $\pm$ 2.798 <sup>a</sup>	76.903 $\pm$ 0.775 <sup>ba</sup>	29.691 $\pm$ 1.767 <sup>c</sup>	381.913	<0.001
<i>n</i> -Octadecane ( <i>n</i> -C <sub>18</sub> )	169.702 $\pm$ 4.495 <sup>a</sup>	105.860 $\pm$ 1.067 <sup>b</sup>	49.860 $\pm$ 2.968 <sup>c</sup>	357.822	<0.001
<i>n</i> -Eicosane ( <i>n</i> -C <sub>20</sub> )	155.249 $\pm$ 4.112 <sup>a</sup>	98.299 $\pm$ 0.991 <sup>b</sup>	44.610 $\pm$ 2.655 <sup>c</sup>	368.244	<0.001
<i>n</i> -Docosane ( <i>n</i> -C <sub>22</sub> )	140.145 $\pm$ 3.712 <sup>a</sup>	81.084 $\pm$ 0.817 <sup>b</sup>	34.806 $\pm$ 2.072 <sup>c</sup>	446.351	<0.001
<i>n</i> -Tricosane ( <i>n</i> -C <sub>23</sub> )	24.728 $\pm$ 0.655 <sup>a</sup>	15.762 $\pm$ 0.159 <sup>b</sup>	2.127 $\pm$ 0.127 <sup>c</sup>	826.376	<0.001
<i>n</i> -Tetracosane ( <i>n</i> -C <sub>24</sub> )	14.190 $\pm$ 0.376 <sup>a</sup>	23.463 $\pm$ 0.236 <sup>b</sup>	2.333 $\pm$ 0.139 <sup>c</sup>	1554.525	<0.001
<i>n</i> -Pentacosane ( <i>n</i> -C <sub>25</sub> )	134.964 $\pm$ 3.575 <sup>a</sup>	76.244 $\pm$ 0.768 <sup>ba</sup>	28.056 $\pm$ 1.670 <sup>c</sup>	532.276	<0.001
<i>n</i> -Hexacosane ( <i>n</i> -C <sub>26</sub> )	54.823 $\pm$ 1.452 <sup>a</sup>	26.394 $\pm$ 0.266 <sup>b</sup>	4.817 $\pm$ 0.287 <sup>c</sup>	834.568	<0.001
<i>n</i> -Heptacosane ( <i>n</i> -C <sub>27</sub> )	107.842 $\pm$ 2.856 <sup>a</sup>	62.660 $\pm$ 0.632 <sup>b</sup>	19.950 $\pm$ 1.187 <sup>c</sup>	581.469	<0.001
<i>n</i> -Octacosane ( <i>n</i> -C <sub>28</sub> )	48.073 $\pm$ 1.273 <sup>a</sup>	64.653 $\pm$ 0.652 <sup>b</sup>	16.860 $\pm$ 1.003 <sup>c</sup>	578.732	<0.001
<i>n</i> -Nonacosane ( <i>n</i> -C <sub>29</sub> )	71.758 $\pm$ 1.901 <sup>a</sup>	50.316 $\pm$ 0.507 <sup>b</sup>	15.671 $\pm$ 0.933 <sup>c</sup>	507.014	<0.001
<i>n</i> -Triacontane ( <i>n</i> -C <sub>30</sub> )	193.154 $\pm$ 5.116 <sup>a</sup>	126.437 $\pm$ 1.274 <sup>b</sup>	32.072 $\pm$ 1.909 <sup>c</sup>	625.086	<0.001
<i>n</i> -Hentriacontane ( <i>n</i> -C <sub>31</sub> )	65.533 $\pm$ 1.736 <sup>a</sup>	47.634 $\pm$ 0.480 <sup>b</sup>	15.115 $\pm$ 0.900 <sup>c</sup>	483.659	<0.001
<i>n</i> -Dotriacontane ( <i>n</i> -C <sub>32</sub> ) Branch	–	18.356 $\pm$ 0.185 <sup>b</sup>	6.193 $\pm$ 0.369 <sup>c</sup>	1538.144	<0.001
<i>n</i> -Dotriacontane ( <i>n</i> -C <sub>32</sub> )	99.128 $\pm$ 2.625 <sup>a</sup>	371.268 $\pm$ 3.742 <sup>b</sup>	100.550 $\pm$ 5.984 <sup>c</sup>	1299.159	<0.001
<i>n</i> -Tritriacontane ( <i>n</i> -C <sub>33</sub> ) Branch	–	–	0.759 $\pm$ 0.045	–	–
<i>n</i> -Tritriacontane ( <i>n</i> -C <sub>33</sub> )	33.377 $\pm$ 0.884 <sup>a</sup>	82.895 $\pm$ 0.836 <sup>b</sup>	25.941 $\pm$ 1.544 <sup>cb</sup>	744.345	<0.001
<i>n</i> -Tetracontane ( <i>n</i> -C <sub>34</sub> ) Branch	–	71.032 $\pm$ 0.716 <sup>a</sup>	26.083 $\pm$ 1.552 <sup>bb</sup>	1325.268	<0.001
<i>n</i> -Tetracontane ( <i>n</i> -C <sub>34</sub> )	23.162 $\pm$ 0.613 <sup>a</sup>	709.447 $\pm$ 7.151 <sup>b</sup>	235.751 $\pm$ 14.031 <sup>c</sup>	1490.775	<0.001
<i>n</i> -Pentatriacontane ( <i>n</i> -C <sub>35</sub> ) Branch	–	7.411 $\pm$ 0.075 <sup>a</sup>	4.832 $\pm$ 0.288 <sup>b</sup>	481.040	<0.001
<i>n</i> -Pentatriacontane ( <i>n</i> -C <sub>35</sub> )	20.853 $\pm$ 0.552 <sup>a</sup>	95.679 $\pm$ 0.964 <sup>b</sup>	35.925 $\pm$ 2.138 <sup>c</sup>	809.106	<0.001
<i>n</i> -Hexatriacontane ( <i>n</i> -C <sub>36</sub> ) Branch	–	34.167 $\pm$ 0.344 <sup>a</sup>	14.727 $\pm$ 0.877 <sup>b</sup>	993.512	<0.001
<i>n</i> -Hexatriacontane ( <i>n</i> -C <sub>36</sub> )	22.667 $\pm$ 0.600 <sup>a</sup>	180.716 $\pm$ 1.821 <sup>b</sup>	75.833 $\pm$ 4.513 <sup>c</sup>	806.833	<0.001

Within rows and columns means followed by same letters (lower and upper case, respectively) are not significantly different ( $P \geq 0.05$ ) by Tukey's HSD test

## Analysis of *n*-alkanes

One half of the first portion of each crude extract of each kind of crop (cabbage, rapeseed, and Indian mustard) leaves was passed through a column of aluminum oxide (Alcoa, Frankfurt, Germany: F-20 grade) 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 using carbon tetrachloride (CCl<sub>4</sub>) 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<sub>f</sub>* (Retention factor) value (0.86) was compared with the *R<sub>f</sub>* value of a mixture of synthetic *n*-alkanes between *n*-C<sub>10</sub> and *n*-C<sub>40</sub>. 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 using a specified oven temperature program (initially 80 °C held for 2 min, then raised at 15 °C/min to 320 °C, and finally held for 15 min) as described by Roy (2021b). The areas of each peak were converted into quantities of *n*-alkanes based on GC peak area of internal standard heneicosane (*n*-C<sub>21</sub> at 100 ng μl<sup>-1</sup>).

## Analysis of FFAs

Remaining half of the first portion of each crude extract of each crop (cabbage, rapeseed, and Indian mustard) leaves 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 using *n*-butanol:acetic acid:water (4:1:5) as the mobile phase after discarding water. The band (*R<sub>f</sub>* 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 BF<sub>3</sub>-Methanol followed by warming for 5 min in a hot water bath at 50–60 °C and cooled at room temperature of 20–22 °C. 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 Na<sub>2</sub>SO<sub>4</sub>. 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 using

a specified oven temperature program (initially held at 120 °C for 2 min, then raised at the rate of 10 °C/min to 220 °C, and finally held at 220 °C for 15 min) as described by Roy (2021b). The area of each peak was converted into quantities of FFAs based on GC peak area of internal standard methyl tricosanoate (C<sub>23:0</sub> at 100 ng μl<sup>-1</sup>).

## Bioassays

### Wax chemicals for bioassays

Both natural *n*-alkanes and FFAs isolated from leaf surface wax of the three brassicaceous crops (cabbage, rapeseed, and Indian mustard) were prepared in leaf equivalent (μg leaf<sup>-1</sup> ml<sup>-1</sup>) amount dissolving in petroleum ether for different bioassays (olfactory attraction and oviposition) of selected generalists (*S. obliqua* and *P. brassicae*) and specialist (*P. xylostella*) pests through different treatments under defined conditions. Petroleum ether was used as the control solvent because both adults and larvae of the pests were neither attracted nor deterred by it in their preliminary bioassay experiments. The synthetic individual *n*-alkanes, FFAs, and their mixtures mimicking the natural leaf wax (μg leaf<sup>-1</sup> ml<sup>-1</sup>) were prepared by the same procedure as in naturally isolated chemicals. The de-waxed leaves for the bioassays were prepared using fresh leaves before dipped in *n*-hexane for 1 min as described in wax extraction process.

### Insects for bioassays

Newly emerged (1–2 days old) F<sub>4</sub> mature females of *S. obliqua*, *P. brassicae*, and *P. xylostella* 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 treatments. Always healthy individuals were selected and used once throughout the bioassay experiments with three replications for each pest species.

### Y-tube olfactometer for bioassays:

The effectiveness of *n*-alkanes and FFAs as olfactory attractants was evaluated by different treatments under specified conditions described below. The behavioral responses of adult females were investigated in a Y-tube olfactometer [20.0-cm long (l) stem and arms, 8.0 cm diameter (d), 60° Y angle] as described by Roy (2019a). The stem of the olfactometer was connected to a porous glass vial [(8.0 cm (d) × 10.0 cm long (l))] in which test insects were released. Each arm of the olfactometer was connected to a glass micro kit adapter [4.0 cm (d) × 6.0 cm (l)] fitted into a glass vial [8.0 cm (d) × 8.0 cm (l)]. The membrane pump producing an air flow of 450 ml min<sup>-1</sup> was first purified by passing

through charcoal filter and the flow of purified air was adjusted to  $150 \text{ ml min}^{-1}$  which led into left and right glass vials through the micro kit adapters. All the connections between different parts of the set-up consisted of silicon tube. One ml of solvent bearing one gram leaf equivalent ( $\mu\text{g leaf}^{-1} \text{ ml}^{-1}$ ) amount of identified *n*-alkanes and FFAs were applied (individually or in mixture to Whatman no. 41 filter paper pieces [ $4 \text{ cm}^2$ ] or on 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*, *P. brassicae*, and *P. xylostella*) was introduced into the porous glass vial attached with the olfactometer to measure the attractiveness.

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 at 10 cm in each side from the junction 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. 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 ( $24 \times 3$ ) females had used and after testing 12 insects the olfactometer set-up and the position of the two arms were systematically changed (rotated  $180^\circ$ ) in order to avoid any positional biasness.

### Bioassays for host preference by leaf cuticular wax chemicals

The dual-choice tests for olfactory attraction of *S. obliqua*, *P. brassicae*, and *P. xylostella* females to natural *n*-alkanes, FFAs, and wax in leaf equivalent ( $\mu\text{g leaf}^{-1}$ ) amount along with intact leaf of selected three crops (cabbage, rapeseed, and Indian mustard) were conducted to find the most preferred crop for them with three replications in four different treatments under defined conditions as follows:

Condition 1 (C1): Normal vs. de-waxed leaf of the selected crops (ESM Table 2).

Condition 2 (C2): Natural *n*-alkanes treated filter paper vs. solvent (ESM Table 2).

Condition 3 (C3): Natural FFAs treated filter paper vs. solvent (ESM Table 2).

Condition 4 (C4): Natural wax-treated filter paper vs. solvent (ESM Table 2).

The attraction index (AI%) is determined 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 wax chemicals) and *C* is the number of adults (females) attracted in controls (de-waxed leaf or solvent) with few modifications (Singh et al. 2011).

The same dual-choice olfaction test was conducted for identified *n*-alkanes and FFAs present in the most preferred host (cabbage, cv. NS-183) individually through their synthetic analogs in leaf equivalent ( $\mu\text{g leaf}^{-1}$ ) amounts to find the most preferred cues having minimum  $\geq 65\%$  attractiveness with three replications under same conditions as in host preference experiments (ESM Table 3). Even, the same tests to the most preferred cues of selected synthetic *n*-alkanes (*n*-C<sub>16</sub>, *n*-C<sub>18</sub>, *n*-C<sub>20</sub>, *n*-C<sub>22</sub>, *n*-C<sub>22</sub>) and FFAs (C<sub>12:0</sub>, C<sub>16:0</sub>, C<sub>18:3</sub>, C<sub>18:2</sub>, and C<sub>18:1</sub>) were conducted in mixture as well as in combination (5 *n*-alkanes + 5FFAs) under four defined conditions (A1, A2, A3, and A4) with three replications in leaf equivalent ( $\mu\text{g leaf}^{-1}$ ) amounts of respective wax chemicals present in cabbage (cv. NS-183) because they were produced more attractiveness than their individual cues to the pest species (ESM Table 4).

### Female oviposition bioassays

Oviposition preference was assessed using newly emerged 24 pairs of mature male and female of each pest species (*S. obliqua*, *P. brassicae*, and *P. xylostella*) in 3 groups for each ( $24 \times 3 = 72$  pairs) in glass chambers ( $40 \times 40 \times 40 \text{ cm}^3$ ) using the synthetic mixtures as in adult olfactory bioassays (ESM Table 4). 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 interval up to 96 h. For the choice experiments, each filter paper or leaf was marked to create two halves vertically. One half was treated with the test compound and the other half was treated with solvent as a control. Each mixture was applied with a micropipette in leaf equivalent ( $\mu\text{g leaf}^{-1}$ ) amount present in cabbage (cv. NS-183) leaf and after evaporating the solvent, one pair of newly emerged (1–2 days old) F<sub>4</sub> mature adults (1:1 sex ratio) 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 filter paper or leaf 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 through five defined conditions (O1, O2, O3, O4, and O5) for each pest as in adult olfactory attraction (ESM Table 4). The oviposition preference index (OPI%) is determined for the conditions using the formula  $[(I - D)/(I + D) \times 100]$ , where *I* is the number of eggs laid in various treatments and *D* is the number of eggs laid in controls with few modifications (Singh et al. 2011).

## Data analyses

The data on total amounts of *n*-alkanes and FFAs present in the selected hosts (cabbage, rapeseed, and Indian mustard) were in normal distribution (Kruskal–Wallis's test) and analyzed by one-way ANOVA followed by Tukey's HSD test. The data obtained for responses of the selected pests (*S. obliqua*, *P. brassicae*, and *P. xylostella*) to epicuticular wax compounds and mixtures of their synthetic analogs were analyzed by Chi-square ( $\chi^2$ ) test based on the null hypothesis whether the ratio of individual choosing the stimulus vs. the control differ significantly from 1:1 (Zar 1999). Insects that did not respond to any one of the treatments were excluded from the analyses. All the statistical analyses were conducted using the software SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

## Results

### Surface wax

A single mature leaf of cabbage, rapeseed, and Indian mustard yielded  $7948.586 \pm 285.802$ ,  $6594.515 \pm 393.463$ , and  $1528.106 \pm 91.282$   $\mu\text{g}$  (mean  $\pm$  SE,  $n=3$ ) surface wax, respectively (ESM Table 1). The extracted waxes from a single leaf of cabbage, rapeseed, and Indian mustard represented  $1528.620 \pm 40.461$ ,  $2451.319 \pm 24.708$ , and  $836.034 \pm 49.731$   $\mu\text{g}$  *n*-alkanes and  $481.533 \pm 16.555$ ,  $558.142 \pm 17.809$ , and  $139.019 \pm 4.402$   $\mu\text{g}$  FFAs, respectively (ESM Table 1). The extracted *n*-alkanes ( $F_{2,6} = 417.324$ ,  $P < 0.001$ ) and FFAs ( $F_{2,6} = 244.713$ ,

$P < 0.001$ ) of the selected crops were with significant differences (rapeseed > cabbage > Indian mustard) but total waxes were without significant difference ( $F_{2,6} = 1.018$ ,  $P = 0.416$ ) between the crops (cabbage > rapeseed > Indian mustard) at respective leaf equivalent ( $\mu\text{g leaf}^{-1}$ ) amount (ESM Table 1).

### Alkanes in leaf surface wax

Total 25 different *n*-alkanes (20 straight chain + 5 branch chain) were identified between *n*-C<sub>14</sub> to *n*-C<sub>36</sub> and out of them 20, 24, and 25 types of *n*-alkanes were detected from the leaves of cabbage, rapeseed, and Indian mustard, respectively (Table 1). Among them *n*-Triacontane (*n*-C<sub>30</sub>) in cabbage ( $193.154 \pm 5.116$   $\mu\text{g leaf}^{-1}$ ) and *n*-Tetratriacontane (*n*-C<sub>34</sub>) in rapeseed ( $709.447 \pm 7.151$   $\mu\text{g leaf}^{-1}$ ) and Indian mustard ( $235.751 \pm 14.031$   $\mu\text{g leaf}^{-1}$ ) were predominant (Table 1). All the identified *n*-alkanes were differed significantly ( $F_{2,6} \geq 357.822$ ,  $P < 0.001$ ) within the selected crop (cabbage, rapeseed, and Indian mustard) species (Table 1).

### FFAs in leaf surface wax

Total 15 different FFAs (11 saturated + 4 unsaturated) were identified between C<sub>12:0</sub> and C<sub>22:0</sub> and out of them 15, 12, and 13 types of FFAs were detected from the leaves of cabbage, rapeseed, and Indian mustard, respectively (Table 2). Among them hexadecanoic acid (C<sub>16:0</sub>) in cabbage ( $130.905 \pm 4.468$   $\mu\text{g leaf}^{-1}$ ) and hexadecenoic acid (C<sub>16:1</sub>) in rapeseed ( $220.401 \pm 7.199$   $\mu\text{g leaf}^{-1}$ ) and Indian mustard ( $71.969 \pm 2.296$   $\mu\text{g leaf}^{-1}$ ) were predominant (Table 2). All the identified FFAs were differed significantly

**Table 2** Composition of free fatty acids (FFAs) ( $\mu\text{g leaf}^{-1}$ ) in plant surface waxes (Mean  $\pm$  SE,  $n=3$ ) of three selected brassicaceous crops (cabbage, *Brassica oleracea* L. [cv. NS-183], rapeseed, *B. campestris* L. [cv. TS 38], and Indian mustard, *B. juncea* L. [cv. B 85]) determined during their growing season in 2020–2021

Free fatty acids ( $\mu\text{g leaf}^{-1}$ )	Cabbage	Rapeseed	Indian mustard	$F_{2,6}$	$P$
Dodecanoic acid (C <sub>12:0</sub> )	$70.587 \pm 2.409^a$	$2.195 \pm 0.072^b$	$1.684 \pm 0.054^c$	810.816	<0.001
Tridecanoic acid (C <sub>13:0</sub> )	$26.297 \pm 0.898^a$	$7.895 \pm 0.258^b$	$2.512 \pm 0.080^c$	531.225	<0.001
Tetradecanoic acid (C <sub>14:0</sub> )	$7.772 \pm 0.265^a$	$28.503 \pm 0.931^b$	$4.909 \pm 0.157^c$	517.202	<0.001
Pentadecanoic acid (C <sub>15:0</sub> )	$3.418 \pm 0.117^{aA}$	$36.694 \pm 1.198^b$	$4.084 \pm 0.130^c$	740.031	<0.001
Hexadecenoic acid (C <sub>16:1</sub> )	$41.726 \pm 1.424^a$	$220.401 \pm 7.199^b$	$71.969 \pm 2.296^c$	464.060	<0.001
Hexadecanoic acid (C <sub>16:0</sub> )	$130.905 \pm 4.468^a$	$29.559 \pm 0.965^b$	$9.675 \pm 0.309^c$	604.190	<0.001
Heptadecanoic acid (C <sub>17:0</sub> )	$3.468 \pm 0.118^{aA}$	$14.122 \pm 0.461^b$	$0.277 \pm 0.009^c$	695.151	<0.001
Octadecatrienoic acid (C <sub>18:3</sub> )	$36.865 \pm 1.258$	–	–	–	–
Octadecadienoic acid (C <sub>18:2</sub> )	$47.131 \pm 1.609$	–	–	–	–
Octadecenoic acid (C <sub>18:1</sub> )	$75.757 \pm 2.586^a$	–	$2.512 \pm 0.080^b$	830.114	<0.001
Octadecanoic acid (C <sub>18:0</sub> )	$19.743 \pm 0.674^a$	$27.103 \pm 0.885^b$	$4.675 \pm 0.149^c$	311.209	<0.001
Nonadecanoic acid (C <sub>19:0</sub> )	$2.079 \pm 0.071^a$	$37.256 \pm 1.217^b$	$5.933 \pm 0.189^c$	733.917	<0.001
Eicosanoic acid (C <sub>20:0</sub> )	$1.511 \pm 0.052^{aB}$	$25.738 \pm 0.841^b$	$4.193 \pm 0.134^c$	727.665	<0.001
Heneicosanoic acid (C <sub>21:0</sub> )	$1.467 \pm 0.050^{aB}$	$21.235 \pm 0.694^b$	$1.942 \pm 0.062^c$	782.990	<0.001
Docosanoic acid (C <sub>22:0</sub> )	$12.943 \pm 0.442^a$	$107.245 \pm 3.503^b$	$24.694 \pm 0.788^c$	605.472	<0.001

Within rows and columns means followed by same letters (lower and upper case, respectively) are not significantly different ( $P \geq 0.05$ ) by Tukey's HSD test

( $F_{1,4} \geq 311.209$ ,  $P < 0.001$ ) within the selected crop (cabbage, rapeseed, and Indian mustard) species (Table 2).

### Adult olfactory attractions

A series of olfactory bioassays were conducted to find the attractiveness (AI%) of *S. obliqua*, *P. brassicae*, and *P. xylostella* (females) toward leaf cuticular wax chemicals (*n*-alkanes and FFAs) of selected crop (cabbage, rapeseed, and Indian mustard) species, their synthetic individuals, and mixture of most attractive individual wax chemicals in leaf equivalent ( $\mu\text{g leaf}^{-1}$ ) amount (ESM Tables 2, 3 and 4, Figs. 1, 2, 3).

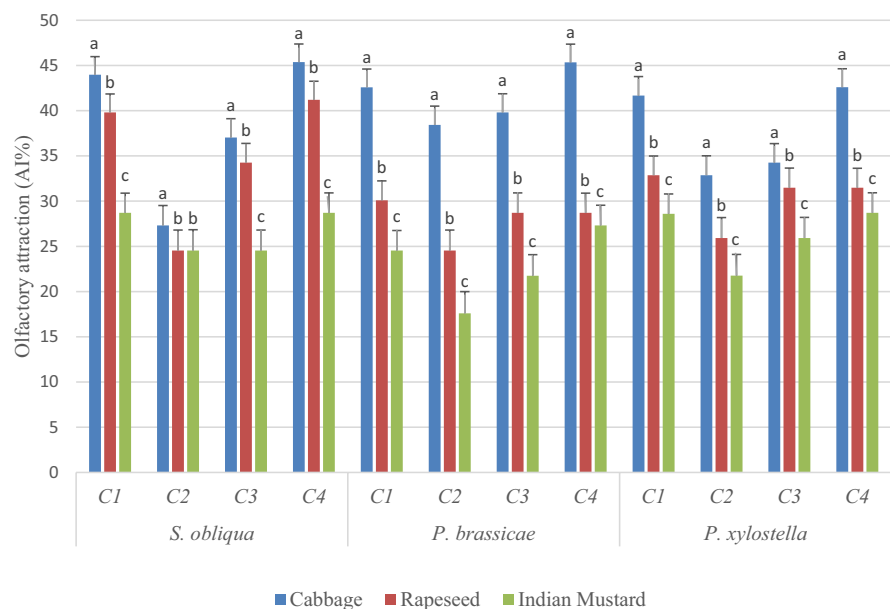
In host preference test highest AI (%) of  $45.370 \pm 2.006$ ,  $45.341 \pm 2.019$ , and  $42.593 \pm 2.045\%$ , respectively, in *S. obliqua*, *P. brassicae* and *P. xylostella* were found toward the natural wax of cabbage leaf ( $7948.586 \pm 285.802 \mu\text{g leaf}^{-1}$ ) which might be due to higher wax content followed by rapeseed and Indian mustard, respectively (ESM Table 2, Fig. 1). The degree of attractiveness (AI%) toward the conditions can be arranged in the order of natural wax (C4) > intact leaf (C1) > FFAs (C3) > *n*-alkanes (C2) for the pests (*S. obliqua* > *P. brassicae* > *P. xylostella*) (ESM Table 2, Fig. 1). The attraction (%) toward any treatments over controls were always significantly ( $\chi^2 \geq 3.874$ ,  $\text{df} = 1$ ,  $P < 0.05$ ) higher in all conditions for each pest species (ESM Table 2). The AI (%) values of the pests were significantly ( $F_{2,6} \geq 7.737$ ,  $P \leq 0.022$ ) differed except C2 and C3 for *S. obliqua* and *P.*

*xylostella*, respectively, within the crop (cabbage > rapeseed > Indian mustard) species (ESM Table 2, Fig. 1).

To find most effective synthetic wax chemicals highest AI (%) was observed toward *n*-hexadecane (*n*-C<sub>16</sub>) and dodecenoic acid (C<sub>12:0</sub>) as  $39.815 \pm 2.578$  and  $43.981 \pm 2.598\%$ , respectively, in *P. xylostella* (ESM Table 3, Fig. 2). Among the treatments the most preferred wax chemicals were consisted of FFAs (C<sub>12:0</sub>, C<sub>16:0</sub>, C<sub>18:3</sub>, C<sub>18:2</sub>, C<sub>18:1</sub>) > *n*-alkanes (*n*-C<sub>16</sub>, *n*-C<sub>18</sub>, *n*-C<sub>20</sub>, and *n*-C<sub>22</sub>, *n*-C<sub>30</sub>) for the three pests (*S. obliqua*, *P. brassicae*, and *P. xylostella*) over the other (ESM Table 3, Fig. 2). The attraction (%) toward individual wax chemicals over controls were always significantly ( $\chi^2 \geq 6.958$ ,  $\text{df} = 1$ ,  $P < 0.05$ ) higher for each pest species except *n*-Triacontane (*n*-C<sub>30</sub>) ( $\chi^2 = 3.032$ ,  $\text{df} = 1$ ,  $P > 0.05$ ) in *P. xylostella* (ESM Table 3). Similarly, all the AI (%) values of selected pests were without significant differences ( $F_{2,6} \leq 4.742$ ,  $P \geq 0.058$ ) for the most preferred wax chemicals except *n*-Triacontane (*n*-C<sub>30</sub>) ( $F_{2,6} = 7.842$ ,  $P = 0.020$ ) within the pests (ESM Table 3, Fig. 2).

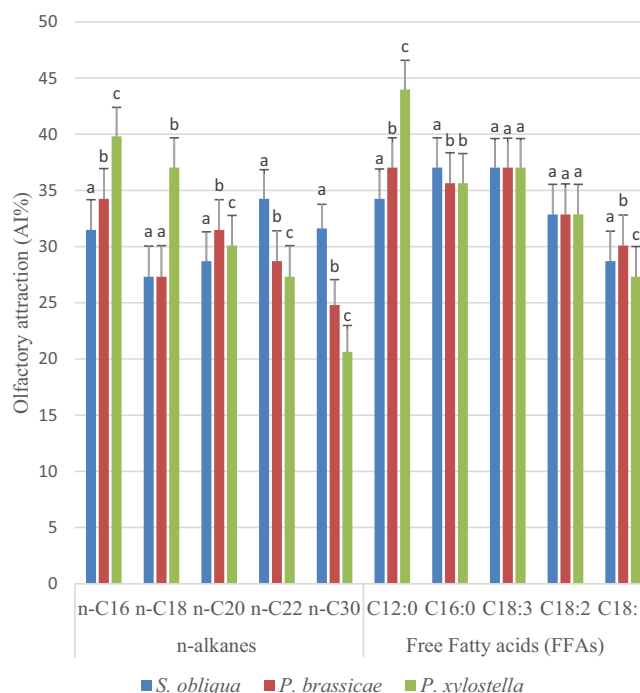
During olfaction toward most effective wax chemicals, the highest AI (%) of  $46.759 \pm 2.377$ ,  $52.315 \pm 2.457$ , and  $49.537 \pm 2.454\%$ , respectively, in *S. obliqua*, *P. brassicae*, and *P. xylostella* were found toward the combined synthetic mixture (5 *n*-alkanes [*n*-C<sub>16</sub>, *n*-C<sub>18</sub>, *n*-C<sub>20</sub>, *n*-C<sub>22</sub>, *n*-C<sub>30</sub>] + 5 FFAs [C<sub>12:0</sub>, C<sub>16:0</sub>, C<sub>18:3</sub>, C<sub>18:2</sub>, and C<sub>18:1</sub>]-treated leaf (A4) of cabbage ( $9073.726 \pm 84.235 \mu\text{g leaf}^{-1}$ ) due to higher amount of wax chemicals followed by combined synthetic mixture-treated filter paper (A3), combined synthetic mixture of

**Fig. 1** Adult olfactory attraction (Mean  $\pm$  SE,  $n = 72$ ) of three lepidopteran pests, polyphagous, *S. obliqua* Walker (Arctiidae), *P. brassicae* L. (Pieridae), and oligophagous, *P. xylostella* L. (Plutellidae) to plant surface wax chemicals (*n*-alkanes and FFAs in leaf equivalent amount [ $\mu\text{g leaf}^{-1}$ ]) of three selected host (cabbage, *B. oleracea* L. [cv. NS-183], rapeseed, *B. campestris* L. [cv. TS 38], and Indian mustard, *B. juncea* L. [cv. B 85]), plants under specified bioassay conditions. Means followed by same letters above the error bars are not significantly different ( $P \geq 0.05$ ) by Tukey's HSD test. Where, C1 intact leaf vs. de-waxed leaf, C2 natural *n*-alkanes treated filter paper vs. solvent, C3 natural free fatty acids (FFAs) treated filter paper vs. solvent, C4 natural wax-treated filter paper vs. solvent, AI Attraction index



Note: Means followed by same letters above the error bars are not significantly different ( $P \geq 0.05$ ) by Tukey's HSD test. Where, C1: Intact leaf vs. de-waxed leaf, C2: Natural *n*-alkanes treated filter paper vs. solvent, C3: Natural free fatty acids (FFAs) treated filter paper vs. solvent, C4: Natural wax treated filter paper vs. solvent, AI=Attraction index.

**Fig. 2** Adult olfactory attraction (Mean  $\pm$  SE,  $n = 72$ ) of three lepidopteran pests, polyphagous, *S. obliqua* Walker (Arctiidae), *P. brassicae* L. (Pieridae), and oligophagous, *P. xylostella* L. (Plutellidae) to plant surface wax chemicals (*n*-alkanes and FFAs in leaf equivalent amount [ $\mu\text{g leaf}^{-1}$ ]) of most preferred host (cabbage, *B. oleracea* L. [cv. NS-183] plants under specified bioassay conditions having more than 65% responses. Means followed by same letters above the error bars are not significantly different ( $P \geq 0.05$ ) by Tukey's HSD test. AI Attraction index



Note: Means followed by same letters above the error bars are not significantly different ( $P \geq 0.05$ ) by Tukey's HSD test. AI=Attraction index.

FFAs (A2), and combined synthetic mixture of *n*-alkanes (A1), respectively (ESM Table 4, Fig. 3). The attraction (%) toward any treatment over controls were always significantly ( $\chi^2 \geq 7.980$ ,  $df = 1$ ,  $P < 0.05$ ) higher for the pests (ESM Table 4). All the AI (%) values were without significant ( $F_{2,6} \leq 4.742$ ,  $P \geq 0.058$ ) differences within the pests (ESM Table 4). In all the treatments AI (%) values for the pests were in the order of *P. brassicae* > *P. xylostella* > *S. obliqua* and in Tukey's HSD test they were with different significant variations (ESM Table 4, Fig. 3).

### Oviposition toward most effective wax chemicals

Highest OPI (%) of  $10.185 \pm 1.234$ ,  $12.963 \pm 1.979$ , and  $15.741 \pm 1.607\%$ , respectively, in *S. obliqua*, *P. brassicae*, and *P. xylostella* were found toward the combined synthetic mixture (5 *n*-alkanes + 5 FFAs)-treated leaf (O5) of cabbage ( $9073.726 \pm 84.235 \mu\text{g leaf}^{-1}$ ) due to higher amount of wax chemicals followed by combined synthetic mixture-treated filter paper (O4), intact leaf (O1), combined synthetic mixture of FFAs (O3), and combined synthetic mixture of *n*-alkanes (O2), respectively (ESM Table 4, Fig. 3). The oviposition choice (%) toward any treatments over controls was always significantly ( $\chi^2 \leq 3.586$ ,  $df = 1$ ,  $P < 0.05$ ) lower except O4 and O5 in *P. xylostella* (ESM Table 4). All the OPI (%) values were without any significant ( $F_{2,6} \leq 2.769$ ,  $P \geq 0.141$ ) differences within the pests as they were all

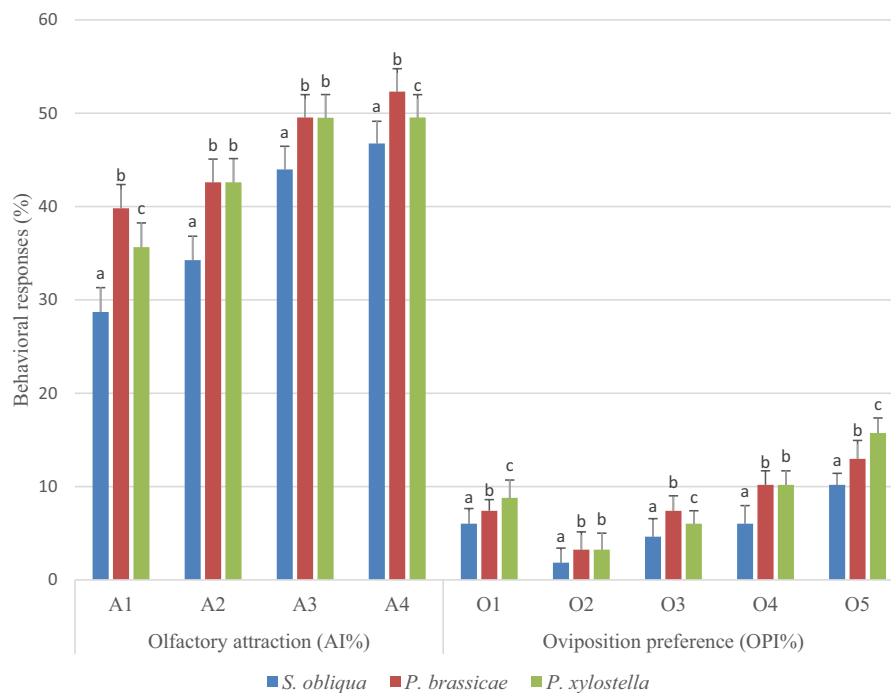
potent pest on cabbage (ESM Table 4). In all the treatments OPI (%) values for the pests were in the order of *P. xylostella* > *P. brassicae* > *S. obliqua* and in Tukey's HSD test they were also with different significant variations (ESM Table 4, Fig. 3).

### Discussion

The epicuticular wax of cabbage, rapeseed, and Indian mustard led through different sensory cues to suitable and differently preferred host selection and oviposition in the polyphagous, *S. obliqua*, *P. brassicae*, and oligophagous, *P. xylostella*, pests like the generalist pests, *Diacrisia casignetum*, *Spilosoma obliqua*, *Helicoverpa armigera*, and *Spodoptera litura* (Roy and Barik 2012, 2014; Roy 2019a, 2021b). Total 25 *n*-alkanes from *n*-C<sub>14</sub> to *n*-C<sub>36</sub> and 15 FFAs from C<sub>12:0</sub> to C<sub>22:0</sub> were detected in leaf epicuticular wax of the three crops as major components with significant variations in their respective quantities ( $\mu\text{g leaf}^{-1}$ ) as in other plants (Mukherjee et al. 2014, 2015; Mitra et al 2017; Das et al. 2019). Among the identified *n*-alkanes and FFAs, *n*-C<sub>34</sub> and C<sub>16:1</sub>, respectively, of rapeseed leaf were most predominant.

Similarly, 9 *n*-alkanes (*n*-C<sub>24</sub> to *n*-C<sub>30</sub>, *n*-C<sub>32</sub>, and *n*-C<sub>33</sub>) and 13 FFAs (C<sub>12:0</sub> to C<sub>20:0</sub>) were detected in the epicuticular wax of mature leaves of sunflower (*H. annuus* cv. PAC-36), where *n*-C<sub>29</sub> and C<sub>18:2</sub>, respectively, were most predominant





Note: Means followed by same letters above the error bars are not significantly different ( $P \geq 0.05$ ) by Tukey's HSD test. Where, A1: Synthetic n-alkanes treated filter paper vs. solvent, A2: Synthetic free fatty acids (FFAs) treated filter paper vs. solvent, A3: Combined synthetic mixture treated filter paper vs. solvent, A4: Combined synthetic mixture treated intact leaf vs. solvent, O1: Intact leaf vs. de-waxed leaf, O2: Synthetic n-alkanes treated filter paper vs. solvent, O3: Synthetic free fatty acids (FFAs) treated filter paper vs. solvent, O4: Combined synthetic mixture treated filter paper vs. solvent, O5: Combined synthetic mixture treated intact leaf vs. solvent, AI=Attraction index, OPI=Oviposition preference index.

**Fig. 3** Olfactory attraction and oviposition preference (Mean  $\pm$  SE,  $n=72$ ) of three lepidopteran pests, polyphagous, *S. obliqua* Walker (Arctiidae), *P. brassicae* L. (Pieridae), and oligophagous, *P. xylostella* L. (Plutellidae) to plant surface wax chemicals (*n*-alkanes and FFAs in leaf equivalent amount [ $\mu\text{g leaf}^{-1}$ ]) of most preferred host (cabbage, *B. oleracea* L. [cv. NS-183]) plants under specified bioassay conditions. Means followed by same letters above the error bars are not significantly different ( $P \geq 0.05$ ) by Tukey's HSD test. Where, A1 synthetic *n*-alkanes treated filter paper vs. solvent, A2 synthetic free

fatty acids (FFAs) treated filter paper vs. solvent, A3 combined synthetic mixture-treated filter paper vs. solvent, A4 combined synthetic mixture-treated intact leaf vs. solvent, O1 intact leaf vs. de-waxed leaf, O2 synthetic *n*-alkanes treated filter paper vs. solvent, O3 synthetic free fatty acids (FFAs) treated filter paper vs. solvent, O4 combined synthetic mixture-treated filter paper vs. solvent, O5 combined synthetic mixture-treated intact leaf vs. solvent, AI attraction index, OPI oviposition preference index

(Roy and Barik 2012, 2014). Eighteen *n*-alkanes (*n*-C<sub>16</sub> to *n*-C<sub>36</sub>) and 13 FFAs (C<sub>12:0</sub> to C<sub>20:0</sub>) were detected in the leaf surface wax of jute (*C. capsularis* cv. Sonali [JRC-321]) and among them *n*-C<sub>29</sub> and C<sub>18:1</sub>, respectively, were most abundant (Roy 2019a). Further, 18 *n*-alkanes (*n*-C<sub>15</sub> to *n*-C<sub>36</sub>) and 14 FFAs (C<sub>12:0</sub> to C<sub>22:0</sub>) were detected in the leaf surface wax of grass pea *Lathyrus sativus*, and, among them, *n*-C<sub>15</sub> and C<sub>15:0</sub>, C<sub>16:1</sub>, respectively, were most predominant (Mitra et al. 2020). Twenty *n*-alkanes (*n*-C<sub>15</sub> to *n*-C<sub>36</sub>) and 13 FFAs (C<sub>12:0</sub> to C<sub>21:0</sub>) were identified from green gram *V. radiata* leaves, and, among them, *n*-C<sub>25</sub> and C<sub>16:1</sub> and C<sub>21:0</sub>, respectively, were most abundant (Mobarak et al. 2020). Twenty *n*-alkanes from *n*-C<sub>14</sub> to *n*-C<sub>36</sub> and 13 FFAs from C<sub>12:0</sub> to C<sub>21:0</sub> was detected in the leaf surface waxes of three *Trichosanthes anguina* L. cultivars (MNSR-1,

Baruipur Long, and Polo No.1) and among them *n*-C<sub>17</sub> and C<sub>18:0</sub> were predominant (Debnath et al. 2021). Cuticular wax of sesame (*Sesamum indicum*) cultivars (Savitri and Nirmala) indicated the presence of 14 *n*-alkanes from *n*-C<sub>9</sub> to *n*-C<sub>44</sub> and 12 FFAs from C<sub>9:0</sub> to C<sub>20:0</sub>, where *n*-C<sub>26</sub> and C<sub>18:1</sub> were most predominant (Roy 2021b). The above-mentioned *n*-alkanes and FFAs can act as short-range attractants for different insect pests to respective host plants (Li and Ishikawa 2006; Malik et al. 2017; Das et al. 2019; Zhu et al. 2021).

Moreover, there are several behavioral studies only on *P. xylostella* other than *P. brassicae* and *S. obliqua* in specific host selection for alternative management strategies (Wee 2016). Host plant-mediated orientation and oviposition by *P. xylostella* were significantly greater on cabbage,

(*B. oleracea* L. subsp. *capitata*) followed by cauliflower (*B. oleracea* L. subsp. *botrytis*), kohlrabi (*B. oleracea* L. subsp. *gongyloides*), and broccoli (*B. oleracea* L. subsp. *italica*) and female oviposition was significantly greater on injured plant leaves than on intact plants leaves (Reddy et al. 2004). Adult *P. xylostella* were equally attracted to transgenic and nontransgenic cabbage plants but larvae were attracted to transgenic plant and suffered heavy mortality (Kumar 2004). The relative roles of olfaction and vision of *P. xylostella* were regulated by plant volatile cues (Couty et al. 2006). Both sexes of *P. xylostella* responded to leaf volatiles of Oriental mustard, *B. juncea* Cosson, and females laid more eggs for the cues (Pivnick et al. 1990). Whereas, *P. brassicae* elicited oviposition-induced plant volatiles having indirect plant defense cues (Fatouros et al. 2015). The electrophysiological and behavioral responses of *P. xylostella*-mated females showed the repellence for five volatile components like myrcene,  $\gamma$ -terpinene, linalool, camphor, and terpinen-4-ol present in none-host geranium plants (Song et al. 2022). The most active green leaf volatile (GLV), (Z)-3-hexenyl acetate, from *B. oleracea* subsp. *capitata* L. enhances attraction to both males and females of *P. xylostella* to the baited traps with pheromone in 1:1 ratio (Reddy and Guerrero 2000). Even, *P. xylostella*-damaged cabbages emitted herbivore-induced volatile compounds like homoterpene (*E*)-4,8-dimethyl-1,3,7-nonatriene, sesquiterpene (*E, E*)- $\alpha$ -farnesene, and (Z)-3-hexenyl acetate and attract their natural enemies for indirect defense of the plants (Vuorinen et al. 2004). Biogenic amines like octopamine and its receptors are involved in mating-triggered oviposition in *P. xylostella* and provide a new strategy for their control (Li et al. 2020). Even, pheromone traps may be a viable alternative to manage *P. xylostella* in an IPM system (Shakeel et al. 2017; Deguine et al. 2021; Passos et al. 2020).

The present study revealed clear olfactory responses of all the pests to *n*-alkanes and FFAs present in leaf cuticular waxes of the selected crops. 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. Even, the role of olfaction is well documented in moths due to their typical nocturnal lifestyle (Cunningham et al. 1999). Visual (Barragán-Fonseca et al. 2020), olfactory (Lucas-Barbosa et al. 2016; Das et al. 2019), tactile (Foster and Howard 1998), 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). Although, resistant cabbage (NY 8329) with glossy leaves having triterpenols  $\alpha$ - and  $\beta$ -amyrin in the waxes reduced acceptance of the neonate *P. xylostella* (Eigenbrode et al. 1991). But, the synergistic effect of sinigrin (10–3, 10–4, and 10–5 M) with *n*-alkanes on *P. xylostella* oviposition behavior was increased by

additional time spend in contact with the stimuli (Spencer et al. 1999). In this study, olfaction (AI%) toward the natural wax (*n*-alkanes and FFAs) for the pests were in the order of *S. obliqua* > *P. brassicae* > *P. xylostella* within the crop (cabbage > rapeseed > Indian mustard) species. The most preferred wax chemicals were consisted of 5 *n*-alkanes (*n*-C<sub>16</sub>, *n*-C<sub>18</sub>, *n*-C<sub>20</sub>, *n*-C<sub>22</sub>, and *n*-C<sub>30</sub>) and 5 FFAs (C<sub>12:0</sub>, C<sub>16:0</sub>, C<sub>18:3</sub>, and C<sub>18:2</sub>, C<sub>18:1</sub>) for the pests. All the AI (%) values of selected pests were without significant differences for the most preferred wax chemicals due to the abundance of host chemicals and preference for the respective hosts as in other pest species (Li and Ishikawa 2006; Mitra et al. 2019). Highest AI (%) in the order of *P. brassicae* > *P. xylostella* > *S. obliqua* was found toward the combined synthetic mixture (5 *n*-alkanes + 5 FFAs)-treated leaf of cabbage due to higher amount of wax chemicals, like other lepidopteran species (Mitra et al. 2020; Mobarak et al. 2020). Similarly, highest OPI (%) was also found toward the same combined synthetic mixture (5 *n*-alkanes + 5 FFAs)-treated leaf of cabbage due to higher amount of wax chemicals as in other pest species (Roy 2019a, 2021b). All the OPI (%) values were without any significant differences within the pests (*P. xylostella* > *P. brassicae* > *S. obliqua*) as they were all potent pest on cabbage. They probably use visual (color and shape), olfactory (odorous *n*-alkanes and FFAs), tactile (surface ultra-structure), and gustatory (cuticular wax) cues in synergistic manner for oviposition preference toward cabbage leaf-like other insects (Mitra et al. 2017; Das et al. 2019; Spencer et al. 1999).

Similarly, 5 long-chain *n*-alkanes (*n*-C<sub>26</sub> to *n*-C<sub>30</sub>) of *Zea mays* L. (Poaceae) and *Fallopia japonica* (Houtt.) Ronse Decr. (Polygonaceae) leaves act as oviposition stimulants in the European corn borer *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) (Udayagiri and Mason 1997; Li and Ishikawa 2006). Further, two FFAs (C<sub>18:1</sub>, C<sub>18:2</sub>) act as host finding and oviposition cues for *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) (Phelan et al. 1991). Fatty acids from C<sub>8:0</sub> to C<sub>12:0</sub>, C<sub>18:1</sub>, and C<sub>18:2</sub> of *Picea* spp. and *Abies* spp. serve as oviposition stimulants for the *Choristoneura fumiferana* (Clemens) (Lepidoptera, Tortricidae) (Grant et al. 2000). Five predominant *n*-alkanes (*n*-C<sub>18</sub>, *n*-C<sub>23</sub>, *n*-C<sub>24</sub>, *n*-C<sub>28</sub>, and *n*-C<sub>32</sub>) and 6 FFAs (C<sub>16:0</sub>, C<sub>16:1</sub>, C<sub>18:0</sub>, C<sub>18:1</sub>, C<sub>18:2</sub>, and C<sub>18:3</sub>) of sunflower-leaf attracted *D. casignetum* (Roy and Barik 2012, 2014). The synthetic combination mixture of 4 *n*-alkanes (*n*-C<sub>17</sub>, *n*-C<sub>18</sub>, *n*-C<sub>27</sub>, and *n*-C<sub>29</sub>) and 5 FFAs (C<sub>16:0</sub>, C<sub>16:1</sub>, C<sub>18:1</sub>, C<sub>18:2</sub>, and C<sub>18:3</sub>) was most attractive to *D. casignetum* adults, whereas the same mixture excluding 2 *n*-alkanes (*n*-C<sub>27</sub>, *n*-C<sub>29</sub>) also caused a significant oviposition preference in jute leaf equivalent amount (Roy 2019a). In the grass pea, 5 *n*-alkanes (*n*-C<sub>15</sub>, *n*-C<sub>22</sub>, *n*-C<sub>25</sub>, *n*-C<sub>27</sub>, and *n*-C<sub>33</sub>) and 2 FFAs (C<sub>13:0</sub>, C<sub>18:2</sub>) in mixture acted as attractants and stimulated the emergence of nymphs in *Aphis craccivora* Koch (Hemiptera: Aphididae) at leaf

equivalent amount (Mitra et al. 2020). In green gram (cv. PDM), 4 *n*-alkanes (*n*-C<sub>25</sub>, *n*-C<sub>27</sub>, *n*-C<sub>29</sub>, and *n*-C<sub>36</sub>) and 3 FFAs (C<sub>16:1</sub>, C<sub>18:0</sub>, and C<sub>18:3</sub>) in combination acted as short-range attractants and oviposition stimulants in females of *S. obliqua* at leaf equivalent amount (Mobarak et al. 2020). A synthetic blend of *n*-C17, *n*-C20, *n*-C26, and C18:0 in one leaf equivalent surface wax of *Trichosanthes anguina* L. (cv. MNSR-1) was acted short-range attractants and oviposition stimulants in *Diaphania indica* (Debnath et al. 2021). The olfactory attraction, oviposition, and feeding preference of 3 generalist pests (*S. obliqua* Walker, *H. armigera* Hübner, and *S. litura* Fabricius) were maximum toward 4 *n*-alkanes (*n*-C16, *n*-C22, *n*-C24, and *n*-C26) and 3 FFAs (C12:0, C14:0, and C18:1) mixture in leaf equivalent amount of sesame (cv. Savitri) cultivar (Roy 2021b).

In this study, the pests (*P. brassicae* > *P. xylostella* > *S. obliqua*) were mostly attracted toward the synthetic blend of 5 *n*-alkanes (*n*-C<sub>16</sub>, *n*-C<sub>18</sub>, *n*-C<sub>20</sub>, *n*-C<sub>22</sub>, and *n*-C<sub>30</sub>) and 5 FFAs (C<sub>12:0</sub>, C<sub>16:0</sub>, C<sub>18:3</sub>, C<sub>18:2</sub>, and C<sub>18:1</sub>) present in the leaf equivalent amount (μg leaf<sup>-1</sup>) of the selected cabbage cultivar. Whereas, the oviposition preference of the pests was in the order of *P. xylostella* > *P. brassicae* > *S. obliqua* due to large amount of wax chemicals along with other physicochemical properties of cabbage leaf which act in synergistic manner as in other studies (Spencer et al. 1999). The specialist (*P. xylostella*) moth has more sensitivity to the leaf cuticular wax chemicals in oviposition site selection than the specialists (*P. brassicae* > *S. obliqua*), like other pests (Akhtar and Isman 2003). Whereas, generalist butterfly (*P. brassicae*) has leaf wax chemical guided more olfaction response than the generalist (*S. obliqua*) and specialist (*P. xylostella*) moths as found in other pests (Pivnick et al. 1994; Schäpers et al. 2015). In other instances, host finding and oviposition site selection require a set of sensory (visual, olfactory, tactile, and gustatory) cues that interact with the females before laying eggs (Dahanukar et al. 2005; Renwick and Chew 1994). Host plant selection for oviposition by the females is a decisive step as they require unique set of recognition cues for suitable host selection in establishing a new generation (Schäpers et al. 2015). The preference–performance hypothesis (PPH) or ‘mother-knows-best’ hypothesis similarly state 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 (Altesor and González 2018; Birke and Aluja 2018). Thus, this finding can explain the clue how mature females of the specialist and generalist pests choose their oviposition site in a perfect fashion for their potential hosts through different sensory modalities for better survival and growth of their neonates, like other insects (Griese et al. 2020; Gripenberg et al. 2010). The females of the selected pests

maximize their own fitness by laying eggs on their preferred crop cultivar (cabbage [cv. NS-183] > rapeseed [cv. TS 38] > Indian mustard [cv. B 85]) where their neonates perform best like other butterflies and moths (Birke and Aluja 2018; Mobarak et al. 2020). So, it can be generalized that both generalist (moth and butterfly) and specialist (moth) have same pattern of olfaction and oviposition preferences with different magnitude of responses. Further, insect herbivores often have higher densities on host plants grown in monocultures than those in diverse environments. Population of *P. xylostella* significantly decreased in a mixed cropping field of *Brassica* spp. due to reduced oviposition compared with a monoculture (Huang et al. 2014). To control *P. xylostella*, the use of intercropping (such as trap crops) is gaining attention as ecologically safe strategy (Hussain et al. 2020). Thus, this study also suggested that the synthetic blends of the 5 *n*-alkanes and 5 FFAs along with the green leaf volatiles (need to determine) of the most preferred cultivar of cabbage (cv. NS-183) can be used as lure to develop baited trap and less preferred crops (rapeseed and Indian Mustard) can be used as main crop with the cabbage as trap crop against the pests for their sustainable ecological management in near future.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s11829-022-09917-w>.

**Acknowledgements** I wish to express my deep sense of gratitude to West Bengal Department of Science and Technology (WBDST) Project [File No.: ST/P/S&T/1G-29/2018], from Government of West Bengal, India for financial assistance. I must acknowledge the farmers who help me in every way during my fieldwork.

**Author contributions** NR designed the whole study including sample collection, chemical analysis, index calculation, data analysis and drafts the manuscript with the help of institutional support.

**Funding** West Bengal Department of Science and Technology (WBDST) Project [File No.: ST/P/S&T/1G-29/2018], from Government of West Bengal, India.

**Data availability** All data generated or analyzed during this study are included in this article.

**Code availability** Not applicable.

## Declarations

**Conflict of interest** The author declares that there is no competing interest other than publication of this paper.

**Ethical approval** Not applicable.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

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