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Interrelation between Surface Wax Alkanes from Red Kidney Bean (*Phaseolus vulgaris* L.) Seeds and Adzuki Bean Weevil [*Callosobruchus chinensis* (F.)] (Coleoptera: Bruchidae)

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ABSTRACT

Background: *Callosobruchus chinensis* (Fabricius) (Coleoptera: Bruchidae) is one of the major insect pests of *Phaseolus vulgaris* L. grains, commonly known as rajma seeds, in Europe and Asia including India. Infestations of these insects destroy majority of legume seeds including rajma which causes a great economic loss. Hence, a proper sustainable pest management measures are necessary for storage of rajma seeds. For this, the study aims to identify and quantify the *n*-alkane profile from the surface waxes of rajma seeds and their role as olfactory cue in *C. chinensis*. Individual synthetic alkane followed by the synthetic blends mimicking rajma seed surface wax *n*-alkanes as olfactory cue was also evaluated.

Methods: Collected rajma seeds were solvent extracted to isolate surface waxes. The extract then fractioned by thin-layer chromatography and followed by gas chromatography-mass spectrometry analyses to purify, quantify and identify *n*-alkanes.

Result: Rajma seeds' surface waxes analysis revealed 18 *n*-alkanes between *n*-C_{15} and *n*-C_{33}. The predominant alkanes were *n*-octacosane and *n*-hexadecane. *n*-Octadecane was the least abundant alkane in seeds. Total alkane content was 3502.67±12.82µg from 100 g (number 200 ± 5.13) seeds. Adult female *C. chinensis* elicited attraction towards the surface wax alkanes at concentrations of 0.5, 1, 2, 4 and 6 seed(s) equivalent of rajma seed(s) in the Y-tube olfactometer bioassay, but the highest attraction was observed at 6 seeds equivalent. Hence, a synthetic alkane blend resembling of 6 seeds equivalent, present in seeds' surface wax alkane or a combination of nine (which elicited positive response) synthetic alkane blend resembling 6 seeds equivalent could be used as lures in developing baited trap in insect pest management programme.

Key words: Alkanes, *Callosobruchus chinensis*, Olfactory bioassay, *Phaseolus vulgaris* seed, Surface wax, Y-tube olfactometer.

INTRODUCTION

Phaseolus vulgaris, commonly known as red kidney bean or rajma bean, belongs to the family Fabaceae. It is a valuable grain legume in Europe, American, tropical and subtropical regions including India (Gepts, 1990; Toro *et al.*, 1990; Dutta *et al.*, 2016). The rajma bean is an important source of dietary protein for millions of people in India (Marwein and Ray, 2019).

It is cultivated in both *Rabi* and *Kharif* seasons throughout India, covering an area of ca. 9.4 million ha (Rawal and Navarro, 2019).

Callosobruchus chinensis (F.) (Coleoptera: Bruchidae) is one of the major destructive pests of legumes including rajma seeds (red kidney bean). Female *C. chinensis* lay eggs on the surface of rajma seeds where the larvae hatch and burrow inside them. After 23-30 days, adults emerge from the seeds passing through the larval and pupal stages (Chakraborty and Mondal, 2015). Infestation of this insect can cause enormous food value loss of legume seeds (nearly 50-60%) (Caswell, 1973). Therefore, proper control measures are necessary for storage of the seeds. Major disinfestants used to control this pest have harmful effects on the environment and human health, for this, the uses of disinfestants have been restricted from 2015 under the policy of Montreal Protocol (United Nations Environment Programme, 1998). Therefore, it is necessary to find alternative methods to control *C. chinensis* as a pest of stored rajma seeds.

Alkanes are the most abundant group of secondary compounds present in most of the plant's or seed's surface wax, though the composition and amount vary among different species (Baker, 1982; Jetter *et al.*, 2000; Nietupski *et al.*, 2005; Shao *et al.*, 2007; Nawrot *et al.*, 2010). For wide range of insects, alkanes act as attractants (Schiestl *et al.*, 1999; Dutton *et al.*, 2000; Roy and Barik, 2012; Sarkar

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et al., 2013a; Mukherjee *et al.*, 2013; Mitra *et al.*, 2019; Das *et al.*, 2019; Mitra *et al.*, 2020) as well as ovipositional stimulants or both (Eigenbrode and Espelie, 1995; Srinivasan *et al.*, 2006; Mitra *et al.*, 2019; Mitra *et al.*, 2020). Long-chain alkanes such as heneicosane, docosane, tricosane, tetracosane, pentacosane, hexacosane and heptacosane elicited attraction to *Andrena nigroaenea* (Kirby) Miller (Schiestl *et al.*, 1999). Pentadecane, octadecane, docosane, pentacosane, heptacosane, octacosane, nonacosane, tritriacontane also display attraction in *Aphis craccivora* females (Mitra *et al.*, 2020). Furthermore, a dose of 14.54, 11.02, 17.99 and 5.92 µg/ml of docosane, tricosane, pentacosane and heptacosane attracts *Lema praeusta* (Fab.) female (Das *et al.*, 2019). D-catechin from the *P. vulgaris* seeds has been shown to act as ovipositional stimulants in *C. chinensis* (Ueno *et al.*, 1990), but till date no information is available on the surface wax alkanes and its role in insect response from rajma seed. For this reason, the *n*-alkane profile from the surface waxes of rajma seeds was identified and quantified and their role as olfactory cue in *C. chinensis* to find the host was studied under laboratory conditions. We also observed the role of individual synthetic alkanes followed by the synthetic blends mimicking rajma seed surface wax alkanes in *C. chinensis* as olfactory cues.

MATERIALS AND METHODS

The whole experiment was performed from June to August, 2019 at the Ecology Research Laboratory, Department of Zoology, Maulana Azad College (West Bengal, India), while the chromatographic analysis was performed in the Chemical-Ecology laboratory, The University of Burdwan, Burdwan, West Bengal, India. Rajma seeds (Arun) were used throughout the study as it is commonly available in market of Burdwan district, West Bengal. Rajma seeds were collected from the market of Burdwan, West Bengal for this study.

n-Alkanes extraction from seed surface

The surface waxes were extracted from 100 g of uninfected and healthy (number 200 ± 5.13) [mean ± standard error (SE)] rajma seeds. One hundred gram seeds were dipped in 2 L *n*-hexane for 1min in room temperature (27°C) for extraction of surface waxes (Barik *et al.*, 2004; Roy *et al.*, 2012a; Sarkar *et al.*, 2013a; Adhikari *et al.*, 2014, Mitra *et al.*, 2020) and the crude extract was filtered through a Whatman (Maidstone, UK) No. 41 filter paper followed by evaporation of the solvent using reduced pressure. The extract was further passed through a column of aluminium oxide (Alcoa, Frankfurt, Germany: F-20 grade) and eluted with petroleum ether. The eluent was then fractioned by TLC on silica gel G (Sigma St. Louis, MO, USA) layers (thickness 0.5 mm) [prepared using a Unoplan coating apparatus (Shandon, London)] with carbon tetrachloride (mobile phase).The TLC plate was air-dried after the appearance of a light yellowish band. The Rf value of this TLC plate was compared and confirmed with the Rf (0.87) value of a standard mixture of alkanes between *n*-C_{15} and *n*-C_{33}. The single yellowish band was eluted from the TLC plate using chloroform. This purified sample does not show presence of any detectable functional groups in IR spectroscopy. The extraction and purification process were repeated three times separately. Half portion of each sample was used for quantification of the alkane compounds by gas chromatography (GC) and identification with coupled gas chromatography-mass spectrometry (GC-MS) while the second portion was used for the olfactory bioassay. GR grade (from E. Merck, India Pvt. Ltd) solvents were used throughout this study.

n-Alkanes quantification by Gas chromatography (GC)

Extracts of rajma seeds (three separate) were analyzed by a Tech comp GC (Em Macau, Rua De Pequim, Nos. 202A-246, Centro Financeiro F7, Hong Kong) model 7900 attached with an HP-1 capillary column (Agilent, USA; length: 30 m × 0.25 mm × 0.25-mm film thickness) and a flame ionization detector (FID). Initially the oven temperature programme was 170°C held for 1 min, then raised at 4°C/ min to 300°C and finally held for 15 min (Barik *et al.*, 2004; Sarkar *et al.*, 2013a; Mukherjee *et al.*, 2013; Adhikari *et al.*, 2014; Das *et al.*, 2019; Mitra *et al.*, 2019; Mitra *et al.*, 2020). The flow rate of carrier gas (nitrogen) was 18.5 ml/min. The volume of sample injected was 1 µl with a split ratio of 1:10. The limit of detection of the GC instrument is $\leq 5 \times 10^{-12}$ g/s (*n*-hexadecane). The peaks were identified by comparing the retention times with those of standard *n*-alkanes from *n*-C_{15} through *n*-C_{33} and the areas of each peak were quantified by using internal standard *n*-tricosane (*n*-C_{23}). Each *n*-alkane (>99% purity) was purchased from Sigma Aldrich (between *n*-C_{15} to *n*-C_{33}).

n-Alkanes identification with coupled gas chromatography-mass spectrometry (GC-MS)

The extracts were also analyzed with an Agilent 6890 GC coupled to a 5973 Mass Selective Detector for confirmation. The temperature programme and column (HP-1) configuration were same as mentioned in GC analysis. The MS parameters were 280°C at the interface, ionization energy 70 eV and scan speed approximately 1 s over the mass range of 40-600 mass units. Helium was the carrier gas. Alkanes were verified by comparing the diagnostic ions and GC retention times with respective standards (between *n*-C_{15} to *n*-C_{33}).

Test insects

Adult *C. chinensis* were collected from the local storehouses containing rajma seeds of Kolkata. They were maintained in glass jars (1 L) covered with fine-mesh nylon nets at 27±1°C temperature, 65 ±10% relative humidity (r. h.) and 12 L:12 D photoperiod in a 'Biological Oxygen Demand' incubator. Newly emerged females (4th generation) were separated from the stock cultures and were kept in separate glass jars without rajma seeds. The behaviour of 90 females

to 0.5,1, 2, 4 and 6 seed(s) equivalent of surface wax alkanes and a control solvent was observed for 3 min in preliminary assays. Virgin females (olfactory responses of virgin or mated females to alkanes odor were same in the preliminary study) were used throughout the olfactory bioassays. Females were used in the bioassays as females are guided by olfactory cues for oviposition on a proper host.

Y-tube olfactory bioassay

Second half of purified alkanes from the surface of rajma seeds were dissolved in petroleum ether to make five different concentrations of 0.5, 1, 2, 4 and 6 seed(s) equivalent for olfactory bioassays. The highest dose of the alkanes was used 6 seeds equivalent for olfactory bioassays as the insect produced the highest significant ($P<0.00001$) attraction. Synthetic mixtures of alkanes were also prepared by maintaining the combinations and amounts of alkane present in the rajma seeds at 0.5, 1, 2, 4 and 6 seed(s) equivalent. Individual synthetic alkanes mimicking the amount present in each concentration of seed surface waxes were used for olfactory bioassays. The behavioural responses of *C. chinensis* females were observed in a glass Y-tube olfactometer (5 cm long stem and arms; 0.6 cm radius and 45° Y-angle between two arms). Each arm of the olfactometer was connected to a glass-made micro kit adapter fitted into a 1 cm radius 3 cm long glass vial. One glass vial contained a piece (1 cm^2) of Whatman No. 41 filter paper added with particular concentration of alkanes, whilst the other glass vial contained a filter paper of same size moistened with petroleum ether (control solvent). An air-flow of 450 ml min^{-1} was passed through charcoal and the charcoal filtered air was pushed into the system at 150 ml min^{-1}. The air was sucked from the porous glass vial at a rate of 100 ml min^{-1}, which was connected to the stem of the olfactometer. Silicon rubber tubes were used to connect different parts of the set-up. To evaluate the role of alkanes as attractant, laboratory condition was maintained at 27± 1°C, r. h. at 70±3% and light intensity at 150 lux. One adult female *C. chinensis* was introduced into the porous glass vial, which was then attached with the stem of the olfactometer and exposed to a particular odor. Insects were not attracted towards the control solvent (petroleum ether) in preliminary assays. The behaviour of each female was observed for 3 min and considered to have made a choice if it reached at the end of either arm. The insect was removed from the Y-tube and the choice made was recorded as a positive response or negative response by one unit, respectively. "No response" was noted when the female remained in the common arm of the Y-tube (Koschier *et al.*, 2000; Mukherjee *et al.*, 2013, 2014; Sarkar *et al.*, 2013a,b; Das *et al.*, 2019; Mitra *et al.*, 2019; Mitra *et al.*, 2020). For experiment with one alkane sample, a group of 90 naïve female insects were used and after testing five insects, the olfactometer set-up was cleaned with petroleum ether followed by acetone. To avoid the positional bias the position of two arms was systematically changed.

Statistical analysis

The data obtained on the responses of *C. chinensis* in olfactometer assays for the different concentrations of seed surface wax alkanes, synthetic mixtures of alkanes and individual synthetic alkanes were analysed by a Chi-square test (Sokal and Rohlf, 1995; Koschier *et al.*, 2000; Roy *et al.*, 2012b; Sarkar *et al.*, 2013a,b; Das *et al.*, 2019; Mitra *et al.*, 2020). Insects that did not respond by selecting either arm of the olfactometer were not included in the analyses.

RESULTS AND DISCUSSION

_n_-Alkane profiles in rajma seed surface

The extraction of 100 g rajma seeds yielded 3.50 ± 0.012 mg of seed surface wax alkanes which is ca. 10% of the total crude extract of surface waxes. Table 1 and Fig. 1 shows 18 *n*-alkanes in the surface waxes of rajma seeds. Octacosane (*n*-C_{28}) was the predominant *n*-alkane, accounting for 846.67±17.64 µg. Heneicosane (*n*-C_{21}) was the second most abundant alkane followed by *n*-tritriacontane (*n*-C_{33}) in the surface waxes of rajma seeds. *n*-Hexadecane (*n*-C_{16}) and *n*-pentadecane (*n*-C_{15}) were the least abundant alkanes in rajma seeds. The rest 13 *n*-alkanes displayed different patterns in the surface waxes of rajma seeds.

Y-tube olfactometer bioassay

The results obtained in the series of olfactometric bioassays showing effectiveness of *C.chinensis* towards alkanes isolated from *P. vulgaris* L. seeds surface waxes are presented in Table 2. Alkanes from the rajma seed surface

Table 1: Amount of alkanes (µg/100 g seeds) in *P. vulgaris* L. seeds surface waxes.

Name of alkanes	Amount of alkanes (µg) (mean ± SE, N = 3)
n-pentadecane (C15)	56.33±3.18
n-hexadecane (C16)	32.33±2.29
n-heptadecane (C17)	65.33±2.03
n-octadecane (C18)	165.67±2.73
n-nonadecane (C19)	252±6.11
n-icosane (C20)	226.67±8.82
n-henicosane (C21)	463.33±8.82
n-docosane (C22)	147±2.31
n-tetracosane (C24)	164±4.36
n-pentacosane (C25)	227.67±8.69
n-hexacosane(C26)	93±5.03
n-heptacosane (C27)	84.33±3.76
n-octacosane (C28)	846.67±17.64
n-nonacosane (C29)	74.33±2.73
n-triacontane (C30)	91±2.31
n-hentriacontane (C31)	157.33±3.28
n-dotriacontane (C32)	58.67±2.85
n-tritriacontane (C33)	297±9.07
Total	3502.67±12.82

attracted the insect significantly at 0.5 seed equivalent (63.33%; χ^2=6.4; df =1; P<0.05), 1 seed equivalent (68.89%; χ^2=12.84; df =1; P< 0.001), 2 seeds equivalent (73.33%; χ^2=19.6; df =1; P< 0.001), 4 seeds equivalent (81.11%; χ^2=34.84; df =1; P<0.00001) and 6 seeds equivalent (86.67%; χ^2=48.4; df =1; P<0.00001). Insects' did not respond towards 0.25 seed equivalent.

Bioassays with the mixtures of synthetic alkanes mimicking the surface wax alkanes of *P. vulgaris* L. seeds are also summarized in Table 2. Insects showed attraction towards a mimic of 0.5 seed equivalent (61.11%; χ^2=4.4; df =1; P<0.05), 1 seed equivalent (66.67%; χ^2=10; df =1; P< 0.01), 2 seeds equivalent (70%; χ^2=14.4; df =1; P< 0.001), 4 seeds equivalent (78.89%; χ^2=30.04; df =1; P<0.00001) and 6 seeds equivalent (84.44%; χ^2=42.71; df =1; P<0.00001).

Table 3 represents the results of olfactory bioassays of *C.chinensis* for individual synthetic alkanes mimicking the surface wax alkanes of *P. vulgaris* L. seeds. The insect responded positively to *n*-C_{21} at 1.16 µg (58.89%; χ^2=4.4; df =1; P=0.09), 2.32 µg (63.33%; χ^2=6.4; df =1; P=0.011), 4.64µg (64.44%; χ^2=7.51; df =1; P<0.01), 9.28 µg (67.78%; χ^2=11.38; df =1; P<0.001) and 13.92 µg (70%; χ^2=14.4; df =1; P<0.001). Females also showed positive responses towards *n*-C_{25} at 0.569 µg (58.89%; χ^2=4.4; df =1; P=0.09), 1.138 µg (64.44%; χ^2=7.51; df =1; P<0.01), 2.276 µg (66.67%; χ^2=10; df =1; P=0.001), 4.552 µg (67.78%; χ^2=11.38; df =1; P<0.001) and 6.828 µg (70%; χ^2=14.4; df =1; P<0.001). A positive response was also recorded for *n*-C_{15} at 0.564 µg (62.22%; χ^2=5.37; df =1; P=0.02) and the response for the same was the highest at 1.692 µg (66.67%; χ^2=10; df =1; P=0.001). Females showed attractions towards *n*-C_{16} at 1.938 µg (60%; χ^2=3.6; df =1; P=0.057), *n*-C_{20} at 1.692 µg (56.67%; χ^2=1.6; df =1; P=0.2), *n*-C_{22} at 2.94 µg (60%; χ^2= 3.6; df =1; P=0.057) and 4.41 µg (62.22%; χ^2=5.37; df =1; P=0.02), *n*-C_{24} at 4.92 µg (61.11%; χ^2=4.4; df =1; P<0.05), *n*-C_{31} at 4.72 µg (61.11%; χ^2=4.4; df =1; P<0.05) and *n*-C_{32} at 1.17 µg (63.33%; χ^2=6.4; df =1; P=0.011) and

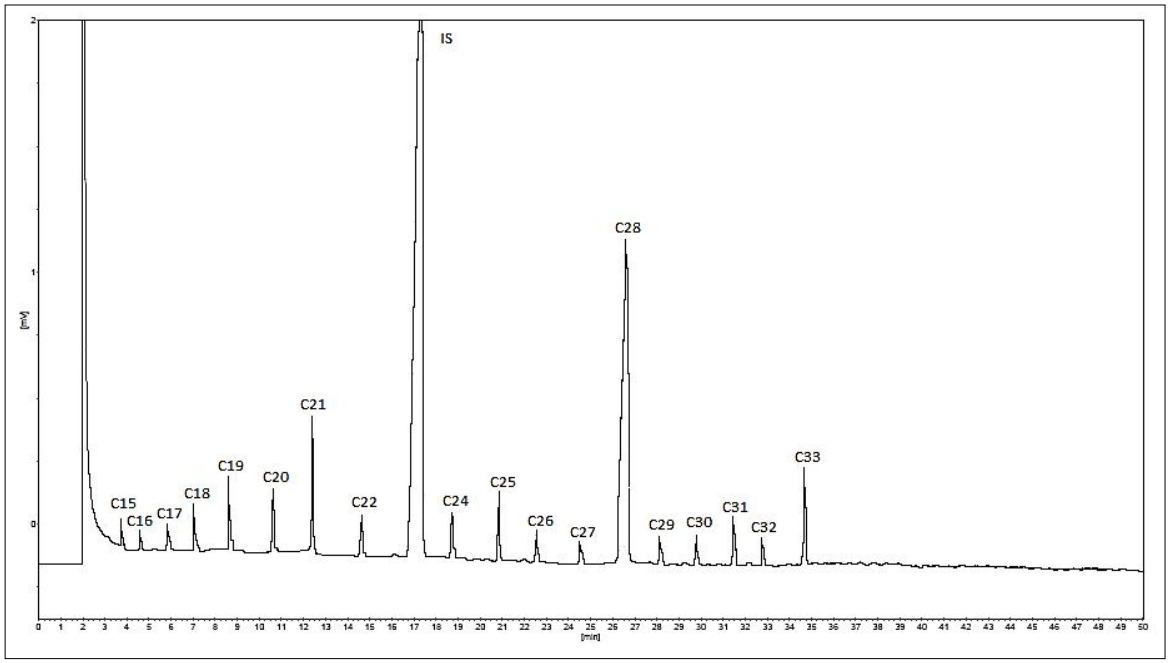

Fig 1: GC-FID chromatogram of rajma seeds surface wax alkanes (IS= Internal Standard, C_X= Carbon chain length).

Table 2: Attractiveness of extracted surface alkanes from *P. vulgaris* L. seeds to *C. chinensis* choosing odour arm (%) in olfactometer bioassay. (N = 90 in each bioassay).

Amount of alkanes used for bioassay	% of insects attracted towards the surface alkanes	% of insects attracted towards the synthetic alkanes mimicking surface waxes
6 seeds equivalent (105 µg)#	86.67%*****	84.44%*****
4 seeds equivalent (70 µg)	81.11%*****	78.89%*****
2 seeds equivalent (35 µg)	73.33%*****	70%***
1 seed equivalent (17.5 µg)	68.89%***	66.67%**
0.5 seed equivalent (8.75 µg)	63.33%**	61.11%*

#The actual amount (µg) of each alkane represented in the bioassay.

*Significant level for p is *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001,*****p<0.00001.

Table 3: Attractiveness of individual and mixtures of synthetic alkanes mimicking the amount present in seed(s) surface waxes of *P. vulgaris* L. to *C. chinensis* choosing odour arm (%) in olfactometer bioassay (N = 90 in each bioassay).

Alkanes	Concentrations				
	0.5 seed equivalent	1 seed equivalent	2 seeds equivalent	4 seeds equivalent	6 seeds equivalent
n-C15 (a)	NR[a](0.141µg)[#]	56.67%(0.28165 µg)	62.22%* (0.564 µg)	64.44%*** (1.128 µg)	66.67%***(1.692 µg)
n-C16 (b)	NR (0.1615 µg)	NR (0.323 µg)	NR (0.646 µg)	55.56% (1.292 µg)	60%* (1.938 µg)
n-C20 (c)	NR (0.5665 µg)	NR (1.133 µg)	NR (2.266 µg)	NR (4.532 µg)	56.67% (6.798 µg)
n-C21 (d)	58.89% (1.16 µg)	63.33%** (2.32 µg)	64.44%** (4.64 µg)	67.78%*** (9.28 µg)	70%**** (13.92 µg)
n-C22 (e)	NR (0.3675 µg)	NR (0.735 µg)	56.67% (1.47 µg)	60%* (2.94 µg)	62.22%** (4.41 µg)
n-C24 (f)	NR (0.41 µg)	NR (0.82 µg)	NR (1.64 µg)	NR (3.28 µg)	61.11% *(4.92 µg)
n-C25 (g)	58.89.% (0.569 µg)	64.44%**(1.138 µg)	66.67%** (2.276 µg)	67.78%*** (4.552 µg)	70%**** (6.828 µg)
n-C31 (h)	NR (0.3935 µg)	NR (0.787 µg)	NR (1.574 µg)	55.56% (3.148 µg)	61.11% *(4.722 µg)
n-C32 (i)	NR (0.1465 µg)	NR (0.293 µg)	NR (0.586 µg)	63.33%** (1.172 µg)	67.78%***(1.758 µg)
[a+b+c+d+e+f+g+h+i]	60%	65.56%**	68.89%***	76.67%*****	80%*****

[a]NR= No response

[#] The actual amount (µg) of each alkane represented in the bioassay.

*Significant level for p is *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, *****p<0.00001.

1.76 µg (67.78%; χ^2=11.38; df =1; P<0.001). Rest of the alkanes did not show attraction in the bioassays. A combination of nine alkane's mixture mimicking the amounts present in the seed surface waxes showed 65.56, 68.89, 76.67 and 80% attraction at 1 seed equivalent (χ^2=8.71; df =1; P<0.01), 2 seeds equivalent (χ^2=12.84; df =1; P<0.001), 4 seeds equivalent (χ^2=25.6; df =1; P<0.00001) and 6 seeds equivalent (χ^2=32.4; df =1; P<0.00001), respectively.

Alkanes are the most common component in plant and seed surface waxes. They have different roles in plant insect interactions such as attractants for feeding (Dutton *et al.*, 2000; Tasin *et al.*, 2005; Sarkar *et al.*, 2013a; Mukherjee *et al.*, 2013; Sarkar and Barik, 2014; Mitra *et al.*, 2019) or stimulants for oviposition (Eigenbrode and Espelie, 1995; Li and Ishikawa, 2006; Das *et al.*, 2019; Mitra *et al.*, 2020). A study by Parr *et al.* (1998) on chickpea seed surface wax showed that heptacosane (*n*-C_{27}) and nonacosane (*n*-C_{29}) were the most abundant *n*-alkanes. *n*-Alkanes with chain lengths from *n*-C_{15} to *n*-C_{32} were present in the surface waxes of khesari seeds among four variety. Further, *n*-C_{19} was the most predominant alkane in surface waxes of four varities of khesari seeds (Adhikary *et al.*, 2014). However in our study, *n*-C_{28} was the most predominant alkane in the surface waxes of rajma seeds.

The olfactory bioassay study provides evidence that the long-chain alkanes can act as close range attractant for *C.chinensis*. Different studies by previous authors demonstrated the importance of surface wax alkanes in different insects (Schiestl *et al.*, 1999; Tasin *et al.*, 2005; Roy and Barik, 2012; Mukherjee *et al.*, 2013; Sarkar *et al.*, 2013a; Adhikary *et al.*, 2014; Mitra *et al.*, 2020). Kotze *et al.* (2010) showed that alkanes from flowers of *Acacia cyclops* A. Cunn.ex G. Don are used by a gall midge, *Dasineura dielsi* Rübsaamen for finding its host. *Aulacophora foveicollis* Lucas females elicited attraction to a synthetic blend of *n*-C_{19}, *n*-C_{27} and *n*-C_{29} alkanes mimicking the amounts present in 6 mg surface wax alkanes of *Momordicacochin chinensis* Spreng flowers (Mukherjee *et al.*, 2013). Adhikary *et al.*, (2014) showed that a blend of *n*-C_{15}, *n*-C_{18}, *n*-C_{19}, *n*-C_{21}, *n*-C_{23} and *n*-C_{25} with the amount of 0.33, 0.20, 1.16, 0.94, 0.75 and 0.56 µg, respectively attracted the *C. maculatus*. *Aphis craccivora* females showed attraction towards a synthetic blend of pentadecane, octadecane, docosane, pentacosane, heptacosane, octacosane, nonacosane resembling the amounts present in the leaf surface waxes of BIO L 212 Ratan (BIO) and Nirmal B-1 (NIR) cultivars of *Lathyrus sativus* (Mitra *et al.*, 2020). In the present study, a clear positive attraction (P<0.01) of the insect was recorded at 0.5 seed equivalent surface wax alkanes from rajma seeds, but the highest attraction was recorded (P<0.00001) at 6 seeds equivalent. In general, our results provide evidences that *C.chinensis*, is highly attracted towards 6 seeds equivalent surface wax alkane of rajma seeds and also a synthetic blend of alkane mixture produced same response (P<0.00001) as 6 seeds equivalent surface wax alkanes. We also identified the specific alkanes and their quantity in the surface wax of rajma seeds and suggest that a synthetic blend of nine alkanes (*n*-C_{15}, *n*-C_{16}, *n*-C_{20}, *n*-C_{21}, *n*-C_{22}, *n*-C_{24}, *n*-C_{25}, *n*-C_{31} and *n*-C_{32}) mimicking 6 seeds equivalent may be applied in lures to develop baited trap to control the outbreak of this insect. Different oviposition-deterrent and toxic effects of various botanicals like *Azadirachta indica, Milletiaie ferrnginea* and *Chrysanthemum cineraraefolium* oil had been already known (Mulatu and Gebremedhin, 2000) but no such compounds are available, which can be used as baited trap to control this insect pest. However, further experiment is needed to evaluate the response of the blend combined with those nine synthetic alkanes towards *C. chinensis* in storage condition.

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