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Aggregation responses of *Cimex hemipterus* F. to semiochemicals identified from their excreta

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ABSTRACT

Background & objectives: Bedbug *Cimex hemipterus* lives in cracks and crevices, and shows aggregation activity in the harbourage containing its fecal matter. Limited information is available on the chemicals influencing this aggregation behaviour in *C. hemipterus*. Possible components responsible for aggregation, if identified and evaluated can be helpful in designing bedbug management.

Methods: Study on the isolation and identification of chemicals from the excreta extract responsible for aggregation has been carried by GC-MS. The compounds identified were evaluated at different concentrations to *C. hemipterus* and their attraction index was determined.

Results: The GC-MS analysis of the excreta extract resulted in identification of 33 different types of semiochemicals such as esters, carboxylic acids, alcohols, aldehydes, ketones and other hydrocarbons. Among these 33 compounds of various groups, the carboxylic acid (*E*)-2-hexenoic acid and the aldehyde (*E*)-2-hexenal found to elicit highest positive aggregation behavioural response in all the stages followed by hexenal.

Interpretation & conclusion: The fecal matter influence the aggregation behaviour in *C. hemipterus*. (*E*)-2-hexenoic acid, hexanoic acid, (*E*)-2-hexenal and hexanal found to exhibit aggregation in various stages of bedbug life cycle. These chemical components can be further exploited for designing and development tool for management of bedbugs.

Key words Aggregation behaviour; bedbugs; GC-MS; hexanal; hexenoic acid

INTRODUCTION

Bedbugs (*Cimex hemipterus*) live in cracks and crevices of sorts such as walls, beds, sitting objects, carpets, electrical switches, cup-boards, wooden articles, and the micro-ecological conditions close to the human being. Although, the population of bedbug has been successfully controlled over past few decades, the present scenario of bedbug infestation is drastically changing. There are reports on record of bedbugs in developed countries considered to be highly hygienic and free from such nuisance pests. The resurgence of bedbugs has been found in various countries like Great Britain[1], Australia[2], Italy[3], Korea[4] and Kuwait[5]. In India, bedbug infestation was also reported from Mumbai[6] and Mangalore[7]. There are various concerns and social issues of the bedbug infestation and agencies have come over for the solution and formulated practices to come out from the menace of bedbug[8–9].

Biting of bedbug is painful causing nuisance and skin reactions, dermatitis, anaemia, pleuritis, *etc*[10]. The available literature hints the role of bedbugs as disease transmitting agent is matter of concern for relapsing fever, typhus, kala-azar and hepatitis B[11–13]. Ecological and local environment in human habitat is diversified and bedbugs have successfully adapted to this surpassing the various means for their control and management. Excessive, inadequate and indiscriminate use of insecticides has lead to development of resistance against insecticide, resurgence, re-emergence and infestation[14–15]. This has prompted for efforts to develop an eco-friendly control method. The exploitation of chemical ecology and behaviour of the bedbug with semiochemical based vector control technology could become part of an integrated pest management. The behaviour of bedbugs often influenced or depend on the sensory physiology[16] and alarm pheromones released by conspecifics[17–20] and besides the alarming behaviour, bedbugs show aggregation responses[21–23].

In the present study, attempts have been made to explore the chemical substances present in the (micro) ecological habitat of bedbug influencing its aggregation behaviour. This will help to understand the chemical ecology of semiochemicals responsible for chemical communication and aggregation among bedbugs. Once the semiochemicals responsible for chemical communication and aggregation are identified, these can be further exploited for management and control of bedbugs resting in the (micro) ecological habitat of the human dwellings. The present study was carried out to identify the source of aggregation factor(s) and identification of chemicals responsible for aggregation of bedbug *C. hemipterus*.

MATERIAL & METHODS

Isolation and identification of aggregation pheromone in C. hemipterus

Studies were carried out to identify the source of chemical components responsible for aggregation and identification of chemicals responsible for aggregation in bedbugs by extraction and isolation of chemicals from the following sources.

The bedbugs were drawn from the stock colony maintained in the laboratory for more than two decades according to Damodar *et al*[24], released on the filter paper in a clean glass chimney used for rearing. The bedbugs were allowed to attach to the filter paper for shelter. Bedbugs were maintained on the filter paper for ten days and the fecal matter released by them during this period was further used for extraction. The extraction was done by methanol solvent of HPLC grade (Fluka/Sigma-Aldhrich). Extraction was done using 15 ml of solvent each separately from filter paper used for 500 bedbugs in a single chamber. The methanol extract of the excreta, of all the stages (nymph, male and female) of bedbugs was analysed by gas chromatography-mass spectrometry (GC-MS) (Agilent Technologies, Germany) to identify its chemical composition. A reconstructed total ion chromatogram (TIC) was obtained by GC-MS of excreta methanol extract, acquired in electron ionisation (EI) mode. Each peak obtained in the chromatogram of excreta extract mass spectra was matched with those of the library database of reference EI mass spectra for tentative identifications of the chemicals. The confirmation of the identified chemicals and identification of 33 compounds was done by analyzing the authentic standards under the same GC-MS conditions. The compounds identified were matched with MS of the standard compounds (Sigma Aldrich) for authenticity and confirmation (Table 1, Fig. 1).

Evaluation of identified semiochemical components

The behavioural response of bedbugs was determined by a choice chamber method essentially used for screening repellents for cockroaches with some modifications[25]. The apparatus was designed in our laboratory to test the biological activity of the various samples. The choice chamber (glass) assembly consists the largest central chamber of about 9 cm height and 2 cm diam to which four small chambers were connected on the four sides

Table 1. Compounds identified from the excreta extract of bedbug *Cimex hemipterus* by GC-MS

S. No.	Compounds	Molecular weight
1.	Acetaldehyde	44.05
2.	Hexanal	100.16
3.	Acetamide	59.07
4	*(E)*-2-hexenal	98.14
5.	2-methyl propanoic acid	88.11
6.	*(E)*-2-hexenol	100.16
7.	3-methylthio-propanal	104.17
8.	*(E)*-heptenal	112.17
9.	Dimethyl trisulfide	126.27
10.	Benzaldehyde	106.12
11.	2-octanone	128.21
12.	2-ethyl-1-hexanol	130.23
13.	Diethylene glycol	106.12
14.	Hexanoic acid	116.16
15.	2-propyl-1-pentanol	130.23
16.	*(E)*-2-octenal	126.20
17.	*(E)*-2-hexenoic acid	114.14
18.	Pyrrolidin-2-one	85.10
19.	2-isopropyl-5-methyl-cyclohexanone (menthone)	154.25
20.	Azulene	128.06
21.	Phenyl acetic acid	136.15
22.	*(E)*-2-octenoic acid	142.20
23.	Tridecane	184.36
24.	Tetradecane	198.39
25.	Tetradecanoic acid	228.37
26.	Isopropyl tetradecanoate	270.45
27.	Methyl hexadecanoate	270.45
28.	Hexadecanoic acid	256.42
29.	9,12-octadecadienaote	294.47
30.	Methyl *(Z)*-9-octadecenoate	296.49
31.	*Cis*-9-octadecenoic acid	282.46
32.	Octadecanoic acid	284.48

separately through a tube of 3 cm length and 1 cm diam. The top of all chambers were closed with a muslin cloth. In the four arm apparatus, two jars contained blank filter papers, one jar contained control filter paper and another one contained treated filter paper. Conditioned filter papers for aggregation activity studies were prepared by impregnating with 500 µl of the identified compounds separately and left at the room temperature until the solvent had completely evaporated. Control filter papers were prepared by impregnating with 500 µl of methanol solvent and allowed to evaporate completely. The impregnated filter papers (treated and solvent control) were used for bioassay immediately. Bedbugs of 30 numbers (males, females and nymphs separately) of same age group taken for bioassay were placed in the central chamber after keeping control and treated papers in the side arms. The number of bedbugs entering different arena were recorded after an interval of 30, 60 and 120 min. There were four replicates in each assay.

The basis for calculating activity was the number of bedbugs visited in both control and treated arena. The aggregation activity was expressed as an attraction index (AI) and calculated as follows:

$$AI = \frac{T - C}{T + C}$$

where, T is the mean number of bedbugs observed in the treated arena and C is the mean number of bedbugs observed in the control arena. The index values fall between +1 and –1, the positive index value denotes more bedbugs are attracted in the treated arena than the control, thus, indicate the attractant property of semiochemical component. On the contrary, more the number of bedbugs in the control will give the negative attraction response. The compounds with attraction index (AI) of + 0.3 and above considered as attractant whereas those with – 0.3 and below are considered as repellents[26].

RESULTS

The aggregation factors present in methanol extract of feces of nymphs, males and females of bedbug, *C. hemipterus* may be the products formed as a result of breakdown of blood meal or may be added in the posterior gut or hind gut before defecation by unknown mechanism[21].

Behavioural responses to aldehydes

Seven aldehyde semiochemical components such as (*E*)-2-hexenal, (*E*)-2-heptenal, hexanal, 3-methylthio-propanal, (*E*)-2-octenal, acetaldehyde and benzaldehyde were evaluated at four different concentrations of variable range against males, females and nymphs of *C. hemipterus*. Nymph, female and male bedbugs showed attraction index of + 0.78, + 0.78 and + 0.72 towards (*E*)-2-hexenal at 100 ppm concentration followed by + 0.66, + 0.77 and + 0.56 respectively at 150 ppm. At the lowest concentration of 25 ppm in females, the attraction index was +0.44 however, repellency was found in nymphs and males with the negative AI, i.e. – 0.15 and – 0.72 respectively (Table 2). Female bedbugs have shown positive attraction towards (*E*)-2-heptenal at 200 ppm and 250 ppm, 3-methylthio-propanal at 100 ppm and (*E*)-2-octenal at 2.5 ppm, whereas in nymphs the attraction index was negative, followed by males and females. Among the seven aldehyde semiochemical components (*E*)-2-hexenal has shown higher positive response in adults and nymphs (Table 2).

Table 2. Attraction indices of aldehyde compounds against female, male and nymphal stages of bedbug *C. hemipterus*

Compounds	Concentration (ppm)	Attraction index		
		Nymph	Female	Male
Aldehydes	25	– 0.15	+ 0.44	– 0.72
(*E*)-2-hexenal	50	+ 0.56	+ 0.64	+ 0.3
	100	+ 0.78	+ 0.78	+ 0.72
	150	+ 0.66	+ 0.77	+ 0.56
(*E*)-2-hexenal	100	– 0.33	– 0.18	– 0.028
	150	– 0.39	– 0.08	– 0.034
	200	– 0.10	+ 0.29	+ 0.006
	250	– 0.38	+ 0.16	– 0.74
Hexanal	12.5	+ 0.16	+ 0.21	+ 0.18
	25	+ 0.29	+ 0.29	+ 0.31
	37.5	+ 0.60	+ 0.67	+ 0.63
	50	– 0.07	– 0.38	– 0.25
3-methylthio-propanal	50	– 0.20	– 0.09	– 0.41
	100	– 0.31	+ 0.05	– 0.57
(*E*)-2-octenal	2.5	– 0.48	+ 0.36	– 0.65
	15	– 0.57	– 0.79	– 0.51
Acetaldehyde	50	– 0.47	+ 0.48	+ 0.43
	100	– 0.14	– 0.47	+ 0.29
Benzaldehyde	50	– 0.44	+ 0.38	– 0.54
	100	– 0.46	+ 0.46	– 0.26

Behavioural responses to carboxylic acids

The GC-MS analysis of methanol extract of the bedbug excreta has shown nine carboxylic acids. (*E*)-2-hexenoic acid has exhibited a concentration dependant positive response while for isobutyric acid and tetradecanoic acid a poor positive response was observed in nymphs and female bedbugs. Besides these, remaining five carboxylic acids, i.e. (*E*)-2-octenoic acid, oleic acid, phenyl acetic acid, hexadecanoic acid and octadecanoic acid were found to elicit a repellent response at all the four different concentrations (Table 3).

Behavioural responses to ketone and alcohol compounds

Among the three ketones, male bedbugs have shown a poor but positive response of + 0.13 at 150 ppm towards 2-octanone while at other concentrations three stages nymph, female and male bedbugs for two components 2-pyrrolidinone and menthone (Table 4). Four alcohol components evaluated at 12.5 ppm of diethylene glycol found to elicit positive response in female bedbugs and in rest of the compounds (*E*)-2-hexenol, diethylene glycol, 2-propyl-1-pentanol and 2-ethyl-2-hexanol and concentrations repelling response was observed (Table 4).

Behavioural responses to ester, hydrocarbons and other compounds

In ester compounds, positive response was found in males at 75 ppm and in nymphs at 200 ppm towards me-

Table 3. Attraction indices of carboxylic acid compounds against nymphal, female and male stages of bedbug *C. hemipterus*

Compounds	Concentration (ppm)	Attraction index		
		Nymph	Female	Male
(*E*)-2-hexenoic acid	25	+ 0.05	+ 0.4	+ 0.18
	50	+ 0.26	+ 0.43	+ 0.39
	75	+ 0.42	+ 0.68	+ 0.57
	100	+ 0.70	+ 0.70	+ 0.65
Hexanoic acid	25	+ 0.35	+ 0.42	+ 0.31
	50	+ 0.61	+ 0.60	+ 0.58
	75	– 0.69	+ 0.15	+ 0.26
	100	– 0.18	– 0.29	– 0.13
(*E*)-2-octenoic acid	50	– 0.38	– 0.25	– 0.15
	100	– 0.09	– 0.22	– 0.64
Isobutyric acid	50	– 0.14	+ 0.21	– 0.62
	100	+ 0.02	– 0.44	– 0.66
Tetradecanoic acid	50	– 0.22	– 0.28	+ 0.18
	100	– 0.23	– 0.24	– 0.57
Oleic acid	300	– 0.05	– 0.51	– 0.22
Phenyl acetic acid	50	– 0.12	– 0.27	– 0.31
	100	– 0.32	– 0.09	– 0.63
Hexadecanoic acid	50	– 0.22	– 0.30	– 0.15
	100	– 0.37	– 0.50	– 0.33
Octadecanoic acid	560	– 0.31	– 0.17	– 0.21
	1120	– 0.22	– 0.30	– 0.39

thyl octadecanoate and isopropyl myristate respectively. Five ester compounds, namely methyl oleate, methyl linoleate, isopropyl myristate, methyl octadecanoate and methyl hexadecanoate except above a repellent response was found against all the three bedbug stages (Table 5). Other than esters, three hydrocarbons and dimethyl trisulphide and acetamide were identified from methanol extract of bedbug excreta by GC-MS. Of the three hydrocarbons, in nymphs poor positive attraction (+ 0.02) was observed for tetradecane on the contrary overall repellency was found (Table 5).

DISCUSSION

Bedbugs live in close vicinity of human beings and though not known to transmit dreaded diseases as of other insects such as mosquito vectors but are suspected to be vectors of diseases[10, 14]. Aggregation is one of the important phenomena of the creatures living in the micro-ecological habitats. To understand their behavioural response it is important to investigate chemical ecology of various semiochemical components released from the place where bedbugs are harboured. In the laboratory observation, it is found that bedbugs are always remaining on the filter paper (the micro-ecological condition) where they are colonized which contains rich amount of fecal matter. Preliminary experiments have shown that the bedbugs show attraction towards these filter papers[21]. To exploit this behaviour for designing and development of suitable ecofriendly measures for the management of bedbugs it is essential to identify and evaluate semiochemical components influencing aggregation of bedbugs. Aboul-Nasr and Erakey[16] found that immobile or akinesis state of bedbugs *C. lectularius* at rest is maintained until any sort of stimuli make induce it to move around however, bedbugs showed no response to excreta on filter paper mixed with undigested blood meal.

In the present investigation, about 33 components of various groups such as aldehyde, carboxylic acid, alco-

Table 4. Attraction indices of ketone and alcohol compounds against nymph, female and male stages of bedbug *C. hemipterus*

Compounds	Concentration (ppm)	Attraction Index		
		Nymph	Female	Male
2-octanone	100	– 0.43	– 0.22	– 0.54
2-pyrrolidinone	50	– 0.23	– 0.22	– 0.43
	100	– 0.28	– 0.23	– 0.44
Menthone	50	– 0.26	– 0.27	– 0.23
	100	– 0.19	– 0.22	– 0.19
(*E*)-2-hexanol	50	– 0.21	– 0.57	– 0.14
	100	– 0.43	– 0.58	– 0.23
Diethylene glycol	50	– 0.06	– 0.48	– 0.57
	100	– 0.48	– 0.70	– 0.57
2-propyl-1-pentanol	50	– 0.22	– 0.12	– 0.55
	100	– 0.17	– 0.10	– 0.09
2-ethyl-1-hexanol	100	– 0.18	– 0.54	– 0.35

Table 5. Attraction indices of ester, hydrocarbons and other compounds against female, male and nymphal stages of bedbug *C. hemipterus*

Compounds	Concentration (ppm)	Attraction index		
		Nymph	Female	Male
Ester	50	– 0.23	– 0.26	– 0.28
Methyl oleate	100	– 0.43	– 0.29	– 0.30
Isopropyl myristate	150	– 0.10	– 0.06	– 0.24
Methyl linoleate	100	– 0.23	– 0.42	– 0.73
Methyl hexadecanoate	50	– 0.20	– 0.47	– 0.42
	100	– 0.38	– 0.27	– 0.52
Methyl octadecanoate	50	– 0.19	– 0.31	– 0.12
	100	– 0.34	– 0.23	– 0.60
Hydrocarbons	50	– 0.32	– 0.47	– 0.55
Tridecane	100	– 0.25	– 0.19	– 0.60
Tetradecane	12.5	+ 0.02	– 0.18	– 0.77
	100	– 0.16	– 0.31	– 0.79
Azulene	50	– 0.30	– 0.11	– 0.06
	100	– 0.34	– 0.13	– 0.28
Other compounds	50	– 0.48	– 0.32	– 0.25
Dimethyl trisulphide	100	– 0.57	– 0.51	– 0.02
Acetamide	50	– 0.52	– 0.05	– 0.38
	100	– 0.43	– 0.41	– 0.48

hol, ketone, ester, hydrocarbons and others have been identified from methanol extract of excreta filter paper (used for rearing) of the bedbugs. Although ample literature is available on the aggregation of bedbugs however, not much attention has been paid to the chemical ecology of semiochemicals from the excreta extract responsible for aggregation in tropical bedbug *Cimex hemipterus*. Adults and larvae of *C. lectularius* when irritated release discharge contents trans-hexe-2-en-1-al and trans-act-2-en-1-al, and butane-2-one, acetaldehyde[17]. In addition to these compounds, volatile emissions from males, females and nymphs have resulted a mixed type of response with multi-functionality nature and (*E*)-2-hexenal and (*E*)-2-octenal are important components of aggregation in *C. lectularius*[27] and alarm behaviour in *C. hemipterus*[19]. In *C. lectularius,* the olfactometer study showed preference of bedbugs towards the bedbug exposed paper than the bedbug volatiles[23, 28] which corroborates our findings on the presence of aggregating semiochemical components in the excreta extract of *C. hemipterus*.

With the present study and available literature it seems the behavioural responses to semiochemicals is complex phenomenon and depends upon various biotic and abiotic factors. Considering this, we are of the opinion that aggregation behavioural response may depend on the density of bedbugs, number of sensilla on the antenna[23, 29–30], olfactory receptors neurons responding[22, 24]. Behavioural manipulation by using semiochemicals or odours released from the fecal matter of conspecifics is a novel approach. The chemical exhibiting strong aggregation responses in bedbug *C. hemipterus* could be helpful in the development of odour baited traps for their management for the benefit of society to get respite from their painful bites.

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REFERENCES

1. Paul J, Bates J. Is infestation with common bedbug is increasing? *British Med J* (Letters) 2000; *320:* 1141.
2. Doggett S, Russell RC. The resurgence of bedbugs, *Cimex* spp (Hemiptera: Cimicidae) in Australia, *Proceedings of the Sixth International Conference on Urban Pests.* In: William H Robinson, Dániel Bajomi, editors. Veszprém, Hungary: OOK-Press Kft 2008; p. 407–25.
3. Giorda F, Guardone L, Mancini M, Accorsi A, Macchioni F, Mignone W. Cases of bedbug (*Cimex lectularius*) infestations in northwest Italy. *Vet Ital* 2013; *49*(4): 335–40.
4. Lee I-Y, Ree H-I, An S-J, Linton AJ, Yong T-S. Reemergence of the bedbug *Cimex lectularius* in Seol, Korea. *Korean J Parasitol* 2008; *46*(4): 269–71.
5. El-Azazy OM, Al-Behbehani B, Abdou NE. Increasing bedbug, *Cimex lectularius*, infestations in Kuwait. *J Egypt Soc Parasitol* 2013; *43*(2): 415–18.
6. *Now bedbugs start bugging railway passengers*. New Delhi: The Times of India 2011. Available from: *http://www.timesofindia. indiatimes. com/city/magalore* (Accessed on Februry 20, 2011).
7. *Bedbugs are keeping us awake*, say WR Motormen. Mumbai: Mirror 2010. Available from: *http://www.mumbaimirror.com/ mumbai/others* (Accessed on July 14, 2010).
8. European Code of Practice Version 2. *Bedbug management*. U.K.: Bedbug Foundation 2012; p. 33.
9. Mona MI. The bugs are back in town: Policy and legislation in light of the bedbug resurgence. *Student Scholarship* 2013; p. 272. Available from: *http://erepository.law.shu.edu/student_ scholarship/272*.
10. Delaunay P, Blanc V, Dandline M, Giudice PD, Franc M, Pomares-Estran C, *et al*. Bedbugs and healthcare associated dermatitis, France. *Emrg Infect Dis* (Letters) 2009; *75*(6): 989–90.
11. Doggett SL, Dwyer DE, Peñas PF, Russell RC. Bedbugs: Clinical relevance and control options. *Clin Microbiol Rev* 2012; *25*(1): 164–92.
12. Jupp PG, McElligott SE, Lecastas G. The mechanical transmission of hepatitis B virus by the common bedbug (*Cimex lectularius* L.) in South Africa. *SA Medical J* 1983; *63:* 77–81.
13. Silverman AL, Li Hua Q, Blow J, Zitron IM, Gordon, Edward DS, Walker C. Assessment of hepatitis B virus DNA and hepatitis C virus RNA in the common bedbug (*Cimex lectularius* L.) and kissing bug (*Rhodnius prolixus*). *Am J Gastroenterol* 2001; *96*(7): 2194–8.
14. Romero A, Potter MF, Potter DA, Haynes K. Insecticide resistance in the bedbug: A factor in the pest's sudden resurgence? *J Med Entomol* 2007; *44*(2): 175–8.
15. Durand E, Cannet A, Berdjane Z, Bruel C, Haouchine D, Delaunay P, *et al*. Infestation by pyrethroid resistant bedbugs in the suburb of Paris, France. *Parasite* 2012; *19:* 381–7.
16. Aboul-Nasr AE, Erakey MAS. On the behavior and sensory physiology of the bedbug *Cimex lectularius* L. to some environmental factors: Chemoreception. *Bull Soc Ent Egypte* 1968; *52:* 353–62.
17. Levinson HZ, Bar Ilan AR. Assembling and alerting scents produced by the bedbug, *Cimex lectularius* L. *Experientia* 1971; *27:* 102–3.
18. Harraca V, Ignell R, Lofstedt C, Ryne C, Characterization of the antennal olfactory system of the bedbug (*Cimex lectularius*). *Chem Senses* 2010; *35:* 195–204.
19. Liedtke HC, Abjornsson K, Harraca V, Knudsen JT, Wallin EA, Hedenstrom E, *et al*. Alarm pheromones and chemical communication in nymphs of the tropical bedbug *Cimex hemipterus* (Hemiptera: Cimicidae). *PloS One* 2011; *6*(3): 1–7.
20. Kilpinen O, Liu D, Adamsen PAS, Real-time measurement of volatile chemicals released by bedbugs during mating activities. *PloS One* 2012; *7*(12): 1–10.
21. Parashar BD, Ganesan K, Sukumaran D, Rao YVS, Veer V, Prakash S. Aggregation activity induced by excreta extracts in *Cimex hemipterus* (Hemiptera: Cimicidae). *Entomon* 2003; *28:* 215–22.
22. Pfiester M. Aggregation and dispersal behavior of the common bedbug, *Cimex Lectularius* L., and a method of detection using

canines. A thesis presented to the graduate school of the University of Florida in partial fulfillment of the requirements for the degree of Master of Science, University of Florida 2008. Available from: *http://ufdc.ufl.edu/UFE0022594/00001*.

23. Weeks ENI, Birkett MA, Cameron MM, Pickett JA, Logan JG. Semiochemicals of the common bedbug, *Cimex lectularius* L. (Hemiptera: Cimicidae), and their potential for use in monitoring and control. *Pest Manag Sci* 2010; *67:* 10–20.
24. Damodar P, Wal YC, Perti SL. A note on laboratory culturing of bedbugs. *Indian J Malariol* 1962; *16:* 227–30.
25. Pandey KS, Mendki MJ, Rao KM, Vaidyanathaswamy R. Synthesis and bioevaluation of alicyclic and heterocyclic alkanoates as cockroach attractants. *Biosci Biotech* Biochem 1995; *59:* 725–7.
26. Kramer WL, Mulla MS. Oviposition attractants and repellents of mosquitoes: Oviposition responses of *Culex* mosquitoes to organic infusions. *Environ Entomol* 1979; *8:* 1111–4.
27. Siljander E, Gries R, Khaskin G, Gries G. Identification of airborne aggregation pheromone of the common bedbug, *Cimex lectularius. J Chem Ecol* 2008; *34*(6): 708–18.
28. Weeks ENI, Logan JG, Birkett MA, Pickett JA, Cameron MM. Tracking bedbugs (*Cimex lectularius*): A study of the effect of physiological and extrinsic factors on the response to bedbug-derived volatiles *J Exp Biol* 2013; *216:* 460–9.
29. Singh RN, Singh K, Prakash S, Mendki MJ, Rao KM. Sensory organs on the body parts of the bedbug *Cimex hemipterus* Fabricius (Hemiptera: Cimicidae) and the anatomy of its central nervous system. *Int J Insect Morphol Embryol* 1996; *25*(1/2): 183–204.
30. Mendki MJ, Prakash S, Parashar BD, Agarwal OP. Distribution of sensilla on antenna and rostrum in nymphs and adults of *Cimex hemipterus* Fabricius (Hemiptera, Cimicidae). *Deutsch Entomol Z* 2013; *60*(2): 213–9.

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