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Attraction of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) to four varieties of *Lathyrus sativus* L. seed volatiles

P. Adhikary, A. Mukherjee and A. Barik*

Ecology Research Laboratory, Department of Zoology, The University of Burdwan, Burdwan – 713 104, West Bengal, India

Abstract

Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) is an important stored grain pest of *Lathyrus sativus* L. (Leguminosae), commonly known as khesari, in India, Bangladesh and Ethiopia. Volatiles were collected from four varieties, i.e., Bio L 212 Ratan, Nirmal B-1, WBK-14-7 and WBK-13-1 of uninfested khesari seeds, and subsequently identified and quantified by gas chromatography mass spectrometry and gas chromatography flame ionization detector analyses, respectively. A total of 23 volatiles were identified in the four varieties of khesari seeds. In Bio L 212 Ratan and WBK-13-1 seeds, nonanal was the most abundant followed by farnesyl acetone; whereas farnesyl acetone was predominant followed by nonanal in Nirmal B-1 and WBK-14-7 khesari seeds. The olfactory responses of female *C. maculatus* toward volatile blends from four varieties of khesari seeds, and individual synthetic compounds and their combinations were examined through Y-shaped glass tube olfactometer bioassays. *Callosobruchus maculatus* showed significant preference for the whole volatile blends from Bio L 212 Ratan seeds compared to whole volatile blends from other three varieties. The insect exhibited attraction to five individual synthetic compounds, 3-octanone, 3-octanol, linalool oxide, 1-octanol and nonanal. A synthetic blend of 448, 390, 1182, 659 and 8114 ng/20 µl methylene chloride of 3-octanone, 3-octanol, linalool oxide, 1-octanol and nonanal, respectively, was most attractive to *C. maculatus*, and this combination might be used for insect pest management program such as baited traps.

Keywords: *Lathyrus sativus*, volatiles, Y-shaped olfactometer bioassay

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Introduction

The bruchid *Callosobruchus maculatus* (F.) is a pest of worldwide importance that causes damage to legumes by feeding and/or oviposition (Utida, 1967, 1972; Messina, 1991; Fox *et al.*, 2010). *Lathyrus sativus* L. (Leguminosae), commonly known as khesari, is one of the important grain legumes in Bangladesh, Ethiopia and India (Gaur & Maloo, 2011; Girma & Korbu, 2012). The crop is cultivated by the farmers of developing countries for production of pulse seeds, which are consumed as food. The adult females lay eggs on the khesari seed surface, and the newly hatched larvae of *C. maculatus* pass through four instars to complete their larval development within 12–16 days and finally the adults emerge from the seeds (Adhikary & Barik, 2012). The heavy infestation by this insect in storage reduces food value of the legume. Hence, it is prerequisite to control outbreaks of this insect. Use of methyl bromide as a fumigant for control of this insect will be discontinued worldwide by 2015 under the terms of Montreal Protocol (United Nations Environment Programme, 1998). Further, use of phosphine gas is also harmful as it causes fire in wet conditions and reacts with copper which ultimately damages electrical fittings in the storage (Mbata, 2004). It is noteworthy that some insecticides such as aluminum phosphide is of no more use, and a strain of

*Author for correspondence
Fax: 0342 2634200
E-mail: anandamaybarik@yahoo.co.in

C. maculatus is tolerant to dimethoate, permethrin, etc. (Bogamuwa *et al.*, 2002). Management of the pest typically entails chemical-based insecticide (pyrethroids, organophosphates) application, which might have potential impacts on non-target organisms and human health. Such concerns result in development of alternate control strategies such as baited traps, which might be incorporated into integrated pest management (IPM) schemes for this pest.

Volatile organic compounds (VOCs) such as saturated and monounsaturated six carbon aldehydes, alcohols and corresponding esters, terpenoids, etc. from the seeds play an important role in seed–insect interaction (Schoonhoven *et al.*, 2005). Some synthetic monoterpenoids, such as *E*-anethole, estragole, *S*-carvone, *L*-fenchone, geraniol, γ-terpinene and *DL*-camphor might act as oviposition deterrent to *C. maculatus* populations including mortality of eggs, first and fourth instar larvae, pupae and adults (Mbata & Payton, 2013). Fatty acids from mung bean seed coat surface waxes have been shown as ovipositional stimulant to *C. maculatus* (Parr *et al.*, 1998), but no information is available on long-range volatiles from khesari seeds, which act as attractants of *C. maculatus*. A cursory review of literature indicate that semiochemicals involved in host location might contribute to novel and sustainable pest management program, such as baited traps. To address this, long-range volatiles from four varieties, i.e., Bio L 212 Ratan, Nirmal B-1, WBK-14-7 and WBK-13-1 of uninfested khesari seeds were collected by push–pull technique, and subsequently identified and quantified by gas chromatography mass spectrometry (GC-MS) and gas chromatography flame ionization detector (GC-FID) analyses, respectively. The behavioral responses of mated *C. maculatus* females to whole volatile blends from four varieties of khesari seeds were examined using a Y-shaped glass tube olfactometer bioassay. We further studied the role of individual synthetic volatile compounds and combinations of synthetic compounds that were characteristic of uninfested khesari seeds as an olfactory cue to *C. maculatus*.

Materials and methods

Seed materials

Twenty bags (each bag contained 500 g uninfested seeds) of each variety (Bio L 212 Ratan, Nirmal B-1, WBK-14-7 and WBK-13-1) of khesari seeds were collected from Pulses and Oilseeds Research Station, Behrampore (24°6′N and 88°15′E), West Bengal, India and taken in this laboratory.

Test insects

Callosobruchus maculatus were collected from local stores containing chickpea seeds at Burdwan (23°16′N, 87°54′E), West Bengal, India. They were maintained in 1 liter glass jars containing chickpea seeds for one generation, which were covered with fine-mesh nylon nets at 12 L: 12 D photoperiod in a 'BOD' incubator (ADS instruments and Tech., Calcutta). Active/inactive male and female forms were determined by flight activity, elytra size and intensity of pigmentation on elytra. Newly emerged F_2 inactive males and females (male: antennae long and deeply serrated, and pygidium uniformly covered with golden setae; female: antennae short and subserrated, and pygidium with a pair of black postero-lateral spots) were separated morphologically from the stock cultures everyday at 9 AM and 9 PM and were kept in separate glass jars without chickpea seeds. For mating, virgin inactive females collected within 12 h of adult emergence were provided with a single virgin inactive male in a 60 mm Petri dish. After a single copulation, inactive females were transferred to a 15 cm (length) × 8 cm (diameter) glass jar containing a small Petri dish (2 cm × 1 cm) with water (Howe & Currie, 1964; Fox, 1993). The behavior of 4–6 day-old mated inactive females was observed in olfactory bioassays. Females were used in the bioassay as the females are guided by olfactory cues for finding of a suitable habitat and host for egg laying (Parr *et al.*, 1998).

Chemicals

HayeSep Q (80–100 mesh), nonyl acetate (grade: nature identical), *trans*-2-Penten-1-ol (95%), 1-hexanol (≥99.5%), α-pinene (≥99%), benzaldehyde (≥99%), 1-heptanol (98%), 1-octen-3-ol (≥98%), 3-octanone (≥98%), 3-octanol (99%), benzyl alcohol (99.8%), linalool oxide (≥97%), 1-octanol (≥99%), linalool (97%), nonanal (95%), 1-nonanol (≥98%), decanal (≥98%), geraniol (98%), 1-decanol (99%), α-humulene (≥96%), 1-tridecanol (97%), 1-tetradecanol (97%), farnesyl acetone (≥90%), geranyl linalool (≥95%) and phytol (≥97%) were purchased from Sigma Aldrich, Germany. Methylene chloride (≤99%) was purchased from Merck Limited, India.

Volatile collections

Four hundred grams of each variety of khesari seeds from each bag were placed in 5-liter glass made Erlenmeyer conical flask separately, and a glass stopper fitted with two 0.5 cm radius glass tubes (the orientation of the glass tubes was in opposite direction) was placed in the mouth of the Erlenmeyer conical flask (Supplementary Figure 1). Charcoal-filtered air was pushed (2 liters min^{-1}) though one glass tube into the Erlenmeyer conical flask and pulled (0.5-liter min^{-1}) through another glass tube which was fitted with a volatile collector trap (150 mm long × 5 mm o.d.) containing 80 mg of HayeSep Q as an adsorbent. Volatiles from all treatments ($N = 5$ replicates for each treatment, i.e., 400 g of each variety of seeds from each bag were used separately for collection of volatiles and a total of five volatile samples were collected separately for each variety) were collected over 10 h from 9 AM to 7 PM.

Volatiles were eluted from the adsorbent washing with 500 µl methylene chloride, and concentrated to 200 µl by a nitrogen flow. One hundred micro liters of each extract were used for olfactory bioassays, and the remaining 100 µl were used for chemical analyses. For olfactory bioassays, 20 µl of an aliquot (equivalent to volatiles released by each variety of khesari seeds in ~1 h) were applied to Whatman No. 41 filter paper (1 cm^2). For quantification through GC, nonyl acetate was added as internal standard (IS), at 20 ng $µl^{-1}$.

Chemical analysis of volatile samples

Five separate volatile samples from each variety of khesari seeds were analyzed with an Agilent 6890 GC coupled with a 5973 Mass Selective Detector with an HP-5 column (Agilent; Palo Alto, CA, USA; length: 30 m × 0.25 mm × 0.25-µm film thickness). The oven temperature program was initially 50 °C held for 3 min, and then raised at 3.75 °C min^{-1} to 240 °C and finally held for 5 min. Helium was the carrier gas. The MS parameters were 250 °C at the interface, ionization energy 70 eV, scan speed approximately 1 s. One µl sample was injected with a split ratio of 1:10. The volatile

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compounds were identified by comparison of mass spectra and retention time with those of authentic standards.

For quantification of identified volatile compounds, the volatiles from each treatment were analyzed by a Techcomp GC (Em Macau, Rua De Pequim, Nos. 202A-246, Centro Financeiro F7, Hong Kong) model 7900 fitted with an HP-5 capillary column and a flame ionization detector, which was run under same temperature conditions as described for GC-MS analysis. The carrier gas was nitrogen with a flow rate of 18.5 ml min^{-1}. The injector port temperature was 280 °C. Components were characterized and quantified against the retention times of authentic standards.

Olfactometer bioassays

The behavioral responses of adult female *C. maculatus* to volatiles were carried out in a glass made Y-shaped olfactometer (15 cm stem and each arm 15 cm long, 0.6 cm radius, 45° Y angle). The stem of the olfactometer was connected to a porous glass vial (1 cm radius × 3 cm long) in which test insect was released. Each arm of the olfactometer was connected to a glass-made micro kit adapter fitted into a glass vial (1 cm radius × 3 cm long). One glass vial contained a piece (1 cm^2) of Whatman No. 41 filter paper moistened with 20 µl of volatiles, whilst the other glass vial contained a filter paper of same size moistened with 20 µl of the control solvent (methylene chloride). Charcoal-filtered air was pushed into each arm of the olfactometer at 200 ml min^{-1}. All the connections between different parts of the set-up consisted of silicon tubing.

The effectiveness of volatiles as attractant was evaluated in the following manner in the laboratory at 27 ± 1 °C, 70 ± 3% relative humidity (RH), and light intensity 150 lux. For each experiment, 20 µl of volatile sample and the control solvent were applied to separate filter paper pieces, allowed to evaporate and introduced into the glass vials before the first insect was released into olfactometer. One adult female *C. maculatus* was introduced into the porous glass vial, which was then attached with the stem of the olfactometer and exposed to a particular odor plus one control. The choice behavior of each female in response to individual synthetic volatile compounds or blend of synthetic volatile compounds was observed for 2 min. This insect was not attracted by the control solvent (methylene chloride) in preliminary assays. The olfactory response of bruchids was recorded as one of the three categories choosing between methylene chloride or the treatment volatiles or 'non-responding' (individuals remained in the common arm of the Y-tube by the end of the observation period) (Magalhães *et al.*, 2012; Mukherjee *et al.*, 2014; Sarkar *et al.*, 2014). Each experiment with one volatile sample was conducted until a total of 90 näive female insects had responded. The olfactometer set-up was cleaned with petroleum ether followed by acetone after testing five insects, and the position of the two arms was systematically changed in order to avoid positional bias. After testing five insects, the filter paper pieces containing volatile sample and control solvent have been replaced with new filter paper pieces with volatile sample and control solvent.

Bioassays

Bioassay 1. Volatiles from uninfested khesari seeds tested against solvent control. Responses of female *C. maculatus* to volatiles collected from four varieties of khesari seeds (A: Bio L 212 Ratan, B: Nirmal B-1, C: WBK-14-7 and D: WBK-13-1), respectively, were tested against solvent control (methylene chloride).

Bioassay 2. VOCs from four varieties of khesari seeds tested against each other. Responses of female *C. maculatus* to volatiles collected from khesari seeds were tested in the following combinations: (A) Bio L 212 Ratan vs. Nirmal B-1; (B) Bio L 212 Ratan vs. WBK-14-7; (C) Bio L 212 Ratan vs. WBK-13-1; (D) Nirmal B-1 vs. WBK-14-7; (E) Nirmal B-1 vs. WBK-13-1; (F) WBK-14-7 vs. WBK-13-1 to find out more attractive khesari seeds.

Bioassay 3. Individual synthetic compounds or synthetic blends of volatile compounds mimicking each variety of khesari seeds tested against solvent control. Individual synthetic compounds detected from 400 g of each variety of khesari seed (µg/10 h) were dissolved in 200 µl methylene chloride, and 20 µl of this odor (equivalent to individual volatiles released by each variety of khesari seeds in ∼1 h) were tested against 20 µl solvent control to find response of the insect.

The individual synthetic compounds that produced response to the insect were combined mimicking each variety of seeds and combinations of synthetic compounds (20 µl volatile odors) (Supplementary Table 1) [Bio L 212 Ratan: 4.48 µg 3-octanone + 3.90 µg 3-octanol + 11.82 µg linalool oxide + 6.59-µg 1-octanol + 81.14 µg nonanal were dissolved in 200 µl methylene chloride and 20 µl of this odor (equivalent to volatiles released by Bio L 212 Ratan khesari seeds in ∼1 h) or Nirmal B-1: 7.31 µg 3-octanone + 1.26 µg 3-octanol +14.59 µg linalool oxide + 10.96 µg 1-octanol + 21.14 µg nonanal were dissolved in 200 µl methylene chloride and 20 µl of this odor (equivalent to volatiles released by Nirmal B-1 khesari seeds in ∼1 h) or WBK-14-7: 4.25 µg 3-octanone + 1.52 µg 3-octanol + 7.16 µg linalool oxide + 7.04 µg 1-octanol + 29.36 µg nonanal were dissolved in 200 µl methylene chloride and 20 µl of this odor (equivalent to volatiles released by WBK-14-7 khesari seeds in ∼1 h) or WBK-13-1: 4.05 µg 3-octanone + 1.29 µg 3-octanol + 6.14 µg linalool oxide + 6.72 µg 1-octanol + 32.37 µg nonanal were dissolved in 200 µl methylene chloride and 20 µl of this odor (equivalent to volatiles released by WBK-13-1 khesari seeds in ∼1 h)] were tested against 20 µl solvent control.

Further, two most abundant volatile compounds (nonanal and farnesyl acetone) in each variety of khesari seeds were tested as a synthetic blend (ng/20 µl) at ratios equivalent to that of each variety of khesari seeds (Supplementary Table 2) against solvent control to observe whether the blend displays any change in insect response than individual synthetic compounds to test insects.

Bioassay 4. Volatiles extracted from Bio L 212 Ratan khesari seeds (most attractive stimulus) vs. individual synthetic compound or a combination of synthetic compounds in the proportions presents in 400 g of Bio L 212 Ratan khesari seeds. Twenty µl volatiles extracted from most attractive khesari seed (Bio L 212 Ratan) were compared with the individual compound (ng/20 µl) or combination of synthetic compounds (ng/20 µl) [equivalent to the proportions detected in 400 g Bio L 212 Ratan khesari seeds in ∼1 h].

Bioassay 5. Dose-dependent responses to five synthetic volatile compounds. Responses of female *C. maculatus* to five compounds were tested at different doses against solvent control (3-octanone or linalool oxide or 1-octanol: 5, 10, 20 and 40 µg were separately dissolved in 200 µl methylene chloride, respectively, and 0.5, 1, 2 and 4 µg/20 µl were used for olfactory bioassays; 3-octanol: 2, 4, 8 and 16 µg were separately dissolved in 200 µl methylene chloride, respectively and 0.2, 0.4, 0.8 and 1.6 µg/20 µl were used for olfactory bioassays; and nonanal: 15, 30, 60 and 120 µg were separately dissolved in 200 µl methylene chloride, respectively, and 1.5, 3, 6 and 12 µg/20 µl were used for olfactory bioassays).

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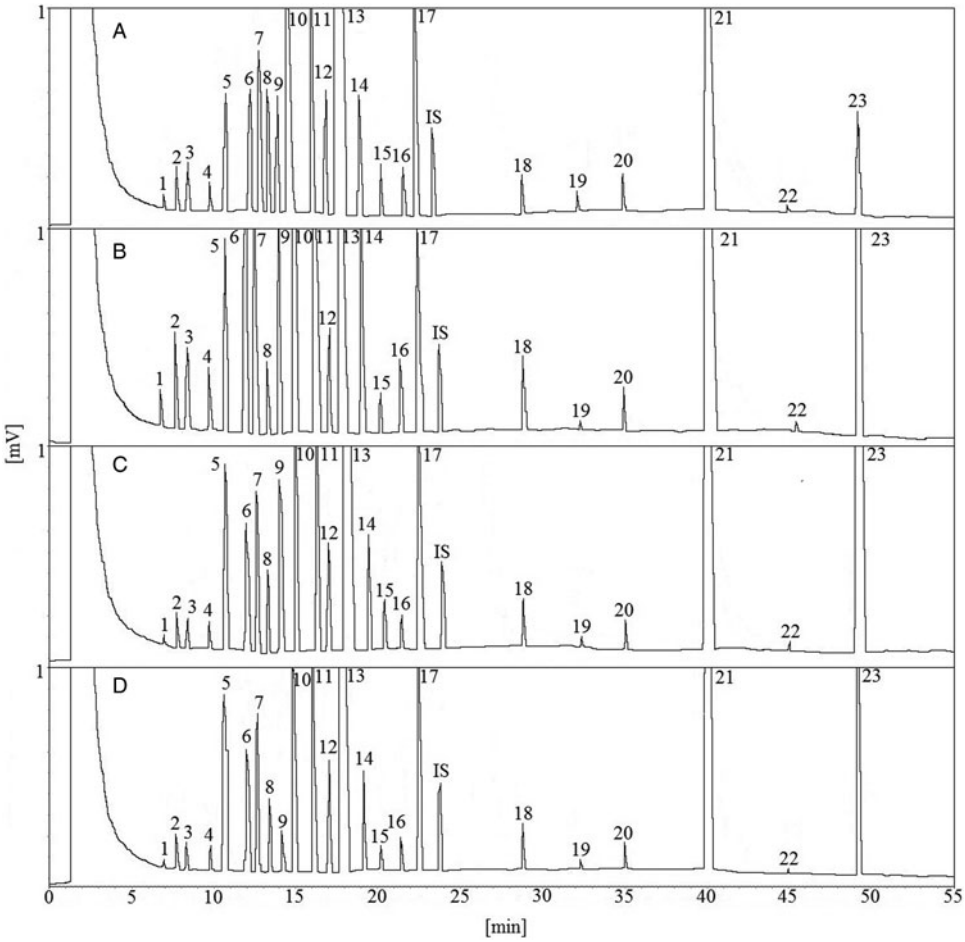

Fig. 1. Example GC-FID chromatogram (HP-5) of volatiles collected from four varieties: (a) Bio L 212 Ratan, (b) Nirmal B-1, (c) WBK14-7 and (d) WBK-13-1 of uninfested khesari seeds. Identification of peaks: 1. *trans*-2-penten-1-ol, 2. 1-hexanol, 3. α-pinene, 4. benzaldehyde, 5. 1-heptanol, 6. 1-octen-3-ol, 7. 3-octanone, 8. 3-octanol, 9. benzyl alcohol, 10. linalool oxide, 11. 1-octanol, 12. linalool, 13. nonanal, 14. 1-nonanol, 15. decanal, 16. geraniol, 17. 1-decanol, 18. α-humulene, 19. 1-tridecanol, 20. 1-tetradecanol, 21. farnesyl acetone, 22. geranyl linalool and 23. phytol.

Statistical analyses

The data on amounts of individual volatiles from each variety of khesari seeds were log $(x + 1)$ transformed prior to performing statistical analysis. In order to highlight the variation in volatile composition within and among the varieties of khesari seeds, a two-way ANOVA was carried out against the individual components as dependent variable and the variety and replicate as explanatory variables. The amounts of individual VOCs present in Bio L 212 Ratan, Nirmal B-1, WBK-14-7 and WBK-13-1 of khesari seeds were subjected to principal component analysis (PCA) to reduce the redundancy of the data of individual volatile compounds and provide ordination of the variables and their each other relationship. Further, in order to interpret the levels of similarity of the volatile compounds in terms of their relative abundance, a cluster analysis (CA) based on Ward's method was carried out using XL STAT software and SPSS version 16. A Kruskal–Wallis nonparametric ANOVA was applied to compare the treatment effects of individual VOCs, and if found significant for Kruskal–Wallis test, the data were analyzed using a Steel–Dwass–Critchlow–Flinger multiple pair wise comparisons test with $\alpha = 0.05$ to find any difference between individual volatile components among four varieties of khesari seeds using XLSTAT software. The log $(x + 1)$ data on total amounts of volatiles from four varieties of khesari seeds were subjected to one-way ANOVA followed by Tukey's test. The data obtained on responses of *C. maculatus* to VOCs were analyzed by a Chi-square (χ^2) test, i.e., probability of scores for the test compound(s) or control solvent is equal to 50% (Roy *et al.*, 2012; Magalhães *et al.*, 2012; Mukherjee *et al.*, 2013; Sarkar *et al.*, 2013*a*, *b*; Sarkar & Barik, 2014). Significance was set to $P < 0.033$ using a Bonferroni correction. Insects that did not respond by selection either arm of the olfactometer were excluded from the analysis.

Results

Chemical analysis

Chemical analyses of volatiles emitted from four varieties, i.e., Bio L 212 Ratan, Nirmal B-1, WBK-14-7 and WBK-13-1 of khesari seeds revealed 23 compounds (fig. 1, table 1). A two-way ANOVA results indicated that for all volatile compounds the *F*-values were consistently non-significant ($P > 0.05$) for replicates (within), while for varieties (among) the *F*-values were

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Table 1. GC-FID analysis of VOCs emitted (μg/10 h) by 400 g uninfested Bio L 212 Ratan, Nirmal B-1, WBK-14-7 and WBK-13-1 *L. sativus* seeds (mean ± SE, $N = 5$).

Peak	Compound	Bio L 212 Ratan	Nirmal B-1	WBK-14-7	WBK-13-1	$X^2_{0.05,\ 3}$	P	Retention time
1	*trans*-2-Penten-1-ol	0.22 ± 0.01^{a}	0.67 ± 0.01^{b}	0.12 ± 0.01^{c}	0.11 ± 0.01^{c}	16.97	0.0007	6.962
2	1-Hexanol	0.82 ± 0.02^{a}	2.04 ± 0.07^{b}	0.82 ± 0.01^{a}	0.78 ± 0.06^{a}	11.05	0.011	7.709
3	α-Pinene	1.00 ± 0.02^{a}	1.89 ± 0.05^{b}	0.70 ± 0.01^{c}	0.65 ± 0.01^{c}	17.90	0.0005	8.331
4	Benzaldehyde	0.59 ± 0.01^{a}	1.22 ± 0.05^{b}	0.47 ± 0.01^{ac}	0.44 ± 0.04^{c}	16.22	0.001	9.758
5	1-Heptanol	4.01 ± 0.08^{a}	5.34 ± 0.11^{b}	4.87 ± 0.06^{c}	4.59 ± 0.13^{c}	16.00	0.0011	10.671
6	1-Octen-3-ol	4.33 ± 0.05^{a}	9.32 ± 0.18^{b}	4.06 ± 0.07^{a}	3.63 ± 0.06^{c}	17.74	0.0005	12.744
7	3-Octanone	4.48 ± 0.07^{a}	7.31 ± 0.08^{b}	4.25 ± 0.10^{c}	4.05 ± 0.08^{c}	14.98	0.0018	13.163
8	3-Octanol	3.90 ± 0.06^{a}	1.26 ± 0.08^{b}	1.52 ± 0.02^{c}	1.29 ± 0.08^{b}	15.94	0.0012	13.360
9	Benzyl alcohol	3.56 ± 0.06^{a}	6.26 ± 0.17^{b}	4.63 ± 0.07^{c}	1.40 ± 0.11^{d}	17.87	0.0005	13.784
10	Linalool oxide	11.82 ± 0.16^{a}	14.59 ± 0.21^{b}	7.16 ± 0.09^{c}	6.14 ± 0.11^{d}	17.86	0.0005	14.440
11	1-Octanol	6.59 ± 0.14^{a}	10.96 ± 0.16^{b}	7.04 ± 0.07^{a}	6.72 ± 0.06^{a}	14.73	0.0021	16.345
12	Linalool	4.31 ± 0.04^{a}	2.07 ± 0.11^{b}	3.12 ± 0.08^{c}	3.31 ± 0.06^{c}	16.64	0.0008	16.953
13	Nonanal	81.14 ± 0.66^{a}	21.14 ± 0.47^{b}	29.36 ± 0.47^{c}	32.37 ± 0.75^{d}	17.58	0.0005	17.379
14	1-Nonanol	3.82 ± 0.03^{a}	7.13 ± 0.09^{b}	3.21 ± 0.05^{c}	2.79 ± 0.05^{d}	17.86	0.0005	18.371
15	Decanal	0.97 ± 0.02^{a}	0.67 ± 0.01^{b}	0.94 ± 0.01^{a}	0.44 ± 0.01^{c}	16.35	0.0010	20.555
16	Geraniol	0.84 ± 0.01^{a}	1.50 ± 0.04^{b}	0.68 ± 0.01^{c}	0.68 ± 0.01^{c}	16.29	0.0010	21.240
17	1-Decanol	6.66 ± 0.22^{a}	5.87 ± 0.18^{b}	6.86 ± 0.16^{a}	6.42 ± 0.13^{ab}	10.02	0.0184	21.461
18	α-Humulene	0.75 ± 0.02^{a}	1.32 ± 0.01^{b}	0.87 ± 0.01^{c}	0.84 ± 0.01^{c}	16.93	0.0007	28.787
19	1-Tridecanol	0.39 ± 0.01^{a}	0.14 ± 0.01^{b}	0.18 ± 0.01^{c}	0.12 ± 0.01^{b}	16.19	0.001	32.375
20	1-Tetradecanol	0.55 ± 0.01^{a}	0.82 ± 0.01^{b}	0.56 ± 0.01^{a}	0.47 ± 0.01^{c}	16.30	0.0010	35.008
21	Farnesyl acetone	46.35 ± 0.95^{a}	47.96 ± 0.67^{a}	34.70 ± 0.55^{b}	22.07 ± 0.38^{c}	16.42	0.0009	39.763
22	Geranyl linalool	0.13 ± 0.01^{a}	0.16 ± 0.01^{b}	0.12 ± 0.01^{a}	0.04 ± 0.01^{c}	17.38	0.0006	45.042
23	Phytol	3.02 ± 0.03^{a}	11.25 ± 0.32^{b}	24.07 ± 0.47^{c}	7.63 ± 0.08^{d}	17.87	0.0005	49.109

X^2value is for Kruskal–Wallis test. Within the row means followed by different letters are significantly different by Steel–Dwass–Critchlow–Flinger multiple pair wise comparisons test with $\alpha = 0.05$. For identification of peaks see fig. 1.

Table 2. The PCA of the dataset on the 23 volatile compounds obtained from four varieties of *L. sativus* seeds.

Communalities	Variables	F1	F2	F3
0.991	*trans*-2-Penten-1-ol	*0.973*	0.123	−0.168
0.976	1-Hexanol	*0.981*	−0.053	−0.103
0.990	α-Pinene	*0.950*	0.242	−0.173
0.962	Benzaldehyde	*0.964*	0.122	−0.135
0.889	1-Heptanol	*0.691*	−0.594	0.241
0.992	1-Octen-3-ol	*0.991*	0.056	−0.075
0.988	3-Octanone	*0.987*	0.047	−0.106
0.972	3-Octanol	−0.336	*0.921*	−0.099
0.989	Benzyl alcohol	*0.758*	0.345	0.543
0.992	Linalool oxide	*0.776*	0.606	−0.146
0.990	1-Octanol	*0.983*	−0.150	−0.045
0.943	Linalool	*−0.824*	0.506	−0.095
0.993	Nonanal	−0.563	*0.797*	−0.204
0.996	1-Nonanol	*0.975*	0.198	−0.083
0.969	Decanal	−0.014	*0.733*	0.657
0.991	Geraniol	*0.965*	0.137	−0.202
0.653	1-Decanol	*−0.644*	0.180	0.453
0.980	α-Humulene	*0.945*	−0.296	−0.012
0.991	1-Tridecanol	−0.292	*0.951*	−0.028
0.968	1-Tetradecanol	*0.973*	0.109	0.095
0.991	Farnesyl acetone	0.631	*0.737*	0.224
0.989	Geranyl linalool	*0.723*	0.562	0.389
0.989	Phytol	0.212	−0.632	*0.738*
	Eigen value	14.615	5.589	1.980
	% of variance	63.543	24.299	8.611
	Cumulative %	63.543	87.842	96.543

The highest loading of the variables for the factors are in italics.

consistently significant ($P < 0.05$) across all the compounds (Supplementary Table 3). This suggested that the variation in volatile composition within a variety was less than among the varieties of khesari seeds considered in this study. The PCA revealed that two factors, F1 and F2, explained more than 87% of the variation in the observed abundance of volatile compounds (table 2, fig. 2). The biplot represented the spatial orientation of the variables against two extracted factors and excepting for phytol, all the variables showed loading for the first two factors (table 2). The communalities of the variables considered were >0.6 with the lowest value shown by 1-decanol. The factor loadings and the contribution of the variables to the factors were shown in table 2. On the basis of the relative abundance per sample, CA allowed classification of 23 volatile compounds under three central classes (fig. 3). Phytol and 1-heptanol representing cluster 1 exhibited greater similarity than that of 1-tridecanol and 1-decanol which represented cluster 3. The relationships of the volatile components in terms of their similarity in relative availability in the khesari seed variety were aptly represented in the cluster and the biplot as well.

For individual VOCs in four varieties of khesari seeds, log $(x + 1)$ transformed data did not reduce the heterogeneity of variances among the groups and, therefore, the original values were used for Kruskal–Wallis nonparametric ANOVA followed by Steel–Dwass–Critchlow–Flinger multiple pair wise comparisons test. Nonanal was predominant followed by farnesyl acetone in Bio L 212 Ratan and WBK-13-1 khesari seeds; whereas farnesyl acetone was predominant followed by nonanal in Nirmal B-1 and WBK-14-7 khesari seeds (table 1). Geranyl linalool was the least abundant in Bio L 212 Ratan and WBK-13-1 khesari seeds; whereas 1-tridecanol was the least abundant in Nirmal B-1 khesari seeds (table 1). Both trans-2-penten-1-ol and geranyl linalool were the least abundant compounds in WBK-14-7 khesari seeds. Rest of the identified VOCs displayed different patterns in four varieties of khesari seeds (table 1). The Levene's test (W) for homogeneity of variance indicated that the data for total amounts of VOCs from four varieties of khesari seeds were homogenous

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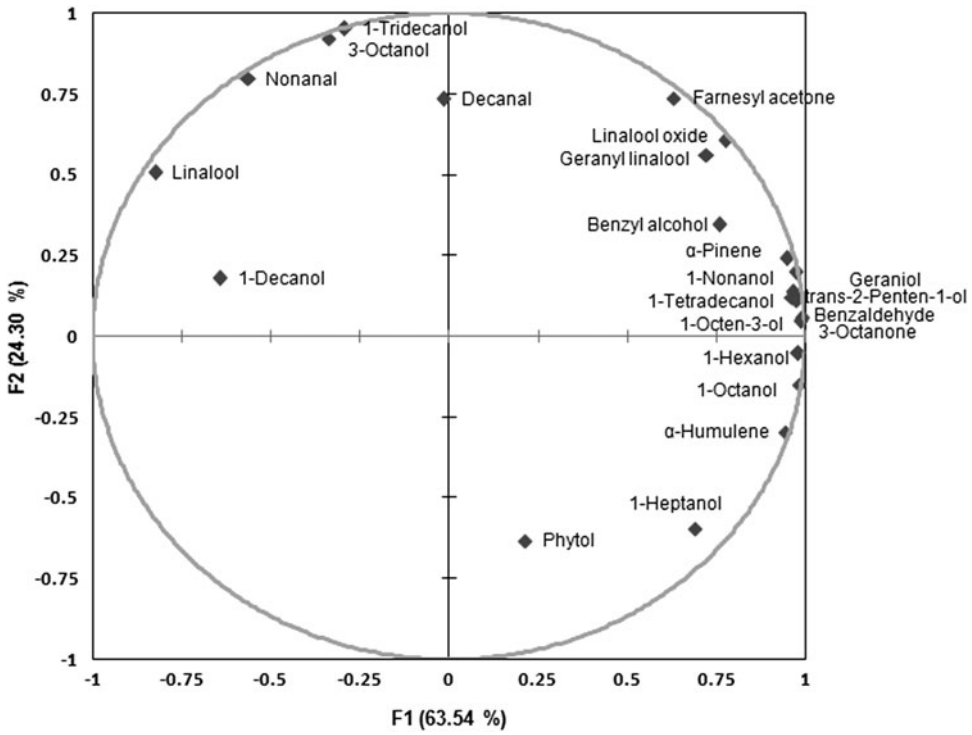

Fig. 2. The biplot showing the ordination of the volatile compounds.

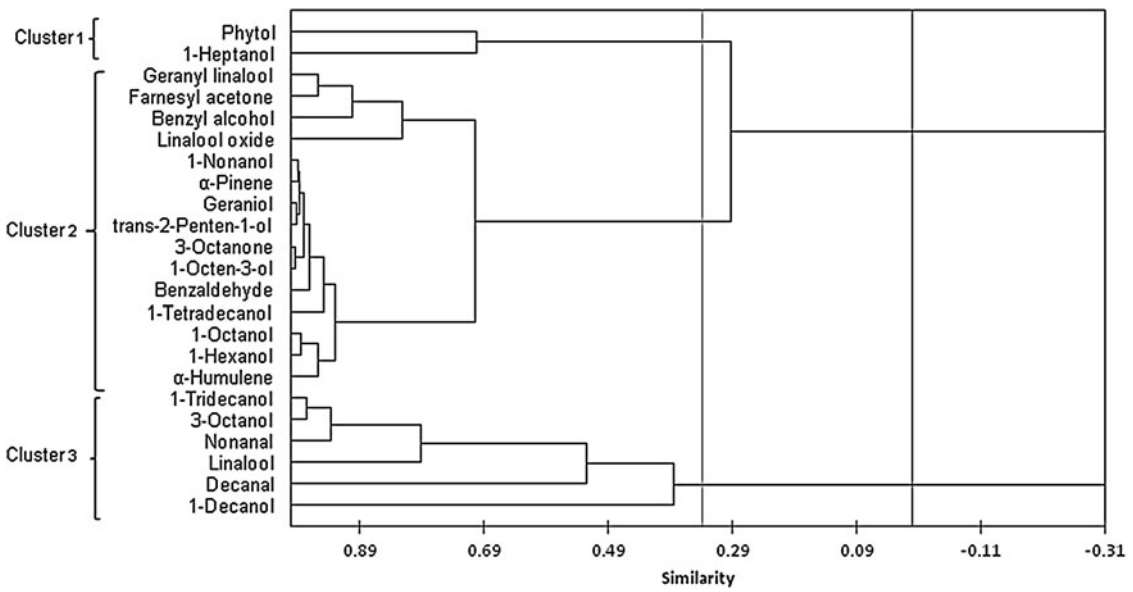

Fig. 3. Dendogram showing relationship among 23 compounds based on their relative abundance in the samples. The similarity is based on Pearson correlation coefficient and the classification is based on unweighted pair-group average.

conforming to application of ANOVA ($W = 0.653$; df = 3, 16; $P > 0.05$). It was observed that the total amounts of VOCs varied significantly with treatments through one-way ANOVA ($F = 915.132$; df = 3, 16; $P < 0.05$), and the Tukey's multiple pair wise comparisons test revealed that total VOCs were significantly higher in Bio L 212 Ratan followed by Nirmal B-1, WBK-14-7 and WBK-13-1 khesari seeds (fig. 4).

Behavioral bioassays

Bioassay 1. Volatiles from uninfested khesari seeds tested against solvent control. In Y-tube olfactometer bioassays, all four varieties of khesari seed types were more attractive than control solvent for female *C. maculatus* (Bio L 212 Ratan: $\chi^2 = 27.78$, df = 1; Nirmal B-1: $\chi^2 = 21.51$, df = 1; WBK-14-7: $\chi^2 = 12.84$, df = 1; WBK-13-1: $\chi^2 = 17.78$, df = 1) (fig. 5).

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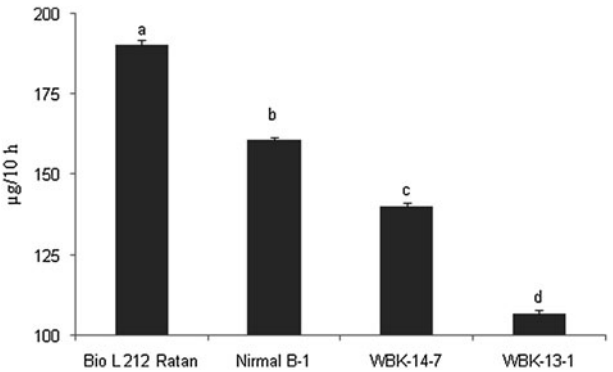

Fig. 4. Total amounts of volatiles (mean ± SE) emitted from four varieties of uninfested khesari seeds.

Bioassay 2. VOCs from four varieties of khesari seeds tested against each other. The insect showed preferences for some varieties over others: Bio L 212 Ratan > Nirmal B-1 > WBK-13-1 and WBK-14-7 [Bio L 212 Ratan seeds ($\chi^2 = 7.51$, df = 1) against Nirmal B-1 seeds; Bio L 212 Ratan seeds ($\chi^2 = 17.78$, df = 1) against WBK-14-7 seeds; Bio L 212 Ratan seeds ($\chi^2 = 11.38$, df = 1) against WBK-13-1 seeds; Nirmal B-1 seeds ($\chi^2 = 6.4$, df = 1) against WBK-14-7 seeds; Nirmal B-1 seeds ($\chi^2 = 5.38$, df = 1) against WBK-13-1 seeds; WBK-13-1 seeds ($\chi^2 = 4.44$, df = 1) against WBK-14-7 seeds] (fig. 6).

Bioassay 3. Individual synthetic compounds or synthetic blends of volatile compounds mimicking each variety of khesari seeds tested against solvent control. Callosobruchus maculatus insects were attracted to five individual synthetic volatile components, 3-octanone, 3-octanol, linalool oxide, 1-octanol and nonanal; whereas rest of the identified 18 individual compounds did not provoke any response to the test insect.

The insect indicated clear positive responses to 3-octanol ($\chi^2 = 6.4$, df = 1), linalool oxide ($\chi^2 = 6.4$, df = 1) and nonanal ($\chi^2 = 14.4$, df = 1) at the proportions equivalent to the Bio L 212 Ratan seeds against solvent control, respectively; whereas the insect did not indicate clear positive and negative responses to 3-octanone ($\chi^2 = 1.6$, df = 1) and 1-octanol ($\chi^2 = 1.6$, df = 1) against solvent control (fig. 7A). The insects displayed attraction to the combination of nonanal and farnesyl acetone like nonanal alone. The insects were attracted to a synthetic blend of five volatile components equivalent to the proportions of five volatile components of Bio L 212 Ratan seeds ($\chi^2 = 21.51$, df = 1) against solvent control (fig. 7A).

The insect displayed attraction to linalool oxide ($\chi^2 = 8.71$, df = 1) at the proportion equivalent to the Nirmal B-1 seeds against solvent control; whereas the insect did not indicate clear positive and negative reactions to 3-octanone ($\chi^2 = 4.44$, df = 1), 3-octanol ($\chi^2 = 0.4$, df = 1), 1-octanol ($\chi^2 = 4.44$, df = 1) and nonanal ($\chi^2 = 1.6$, df = 1) against solvent control (fig. 7B). The insects produced same response to the combination of nonanal and farnesyl acetone like nonanal alone. The insects were attracted to a synthetic blend of five volatile components equivalent to the proportions of five volatile components of Nirmal B-1 seeds ($\chi^2 = 16.04$, df = 1) against solvent control (fig. 7B).

The insect showed attraction to nonanal ($\chi^2 = 5.38$, df = 1) at the proportion equivalent to the WBK-14-7 seeds against solvent control; whereas the insect did not indicate clear positive and negative responses to 3-octanone ($\chi^2 = 1.11$, df = 1), 3-octanol ($\chi^2 = 1.11$, df = 1), linalool oxide ($\chi^2 = 2.84$, df = 1) and 1-octanol ($\chi^2 = 2.18$, df = 1) against solvent control (fig. 7C). The insects displayed same attraction response to the combination of nonanal and farnesyl acetone like nonanal alone. The insects were attracted to a synthetic blend of five volatile components equivalent to the proportions of five volatile components of WBK-14-7 seeds ($\chi^2 = 11.38$, df = 1) against solvent control (fig. 7C).

The insect displayed attraction to nonanal ($\chi^2 = 6.4$, df = 1) at the proportion equivalent to the WBK-13-1 seeds against solvent control; whereas the insect did not indicate clear positive and negative responses to 3-octanone ($\chi^2 = 1.11$, df = 1), 3-octanol ($\chi^2 = 0.4$, df = 1), linalool oxide ($\chi^2 = 1.6$, df = 1) and 1-octanol ($\chi^2 = 1.6$, df = 1) against solvent control (fig. 7D). The insects produced same attraction response to the combination of nonanal and farnesyl acetone like nonanal alone. The insects showed attraction to a synthetic blend of five volatile components equivalent to the proportions of five volatile components of WBK-13-1 seeds ($\chi^2 = 10$, df = 1) against solvent control (fig. 7D).

Bioassay 4. Volatiles extracted from Bio L 212 Ratan khesari seeds (most attractive stimulus) vs. individual synthetic compound or a combination of synthetic compounds in the proportions present in 400 g of Bio L 212 Ratan khesari seeds. The insects were attracted to the volatiles extracted from Bio L 212 Ratan seeds against five individual synthetic compounds in the proportions detected in Bio L 212 Ratan seeds [Bio L 212 Ratan seeds ($\chi^2 = 17.78$, df = 1) vs. 3-octanone, Bio L 212 Ratan seeds ($\chi^2 = 5.38$, df = 1) vs. 3-octanol, Bio L 212 Ratan seeds ($\chi^2 = 5.38$, df = 1) vs. linalool oxide, Bio L 212 Ratan seeds ($\chi^2 = 21.51$, df = 1) vs. 1-octanol, Bio L 212 Ratan seeds ($\chi^2 = 6.4$, df = 1) vs. nonanal]; whereas the insect did not indicate clear positive and negative responses to the volatiles extracted from Bio L 212 Ratan seeds ($\chi^2 = 0.4$, df = 1) against a combination of five synthetic compounds equivalent to the proportions present in Bio L 212 Ratan seeds (fig. 8).

Bioassay 5. Dose-dependent responses to five synthetic volatile compounds. In Y-tube olfactometer bioassays, the insect displayed attraction to 3-octanone, 3-octanol, linalool oxide, 1-octanol and nonanal against solvent control (fig. 9). The insect showed attraction to 3-octanone at 1 µg/20 µl ($\chi^2 = 7.51$; df = 1), 2 µg/20 µl ($\chi^2 = 11.38$; df = 1) and 4 µg/20 µl ($\chi^2 = 19.6$; df = 1) (fig. 9A). The insects were attracted to 3-octanol at 0.4 µg/20 µl ($\chi^2 = 6.4$; df = 1), 0.8 µg/20 µl ($\chi^2 = 12.84$; df = 1) and 1.6 µg/20 µl ($\chi^2 = 23.51$; df = 1) (fig. 9B). The insect produced attraction to linalool oxide at 1 µg/20 µl ($\chi^2 = 6.4$; df = 1), 2 µg/20 µl ($\chi^2 = 11.38$; df = 1) and 4 µg/20 µl ($\chi^2 = 25.6$; df = 1) (fig. 9C). The insect displayed attraction to 1-octanol at 2 µg/20 µl ($\chi^2 = 8.71$; df = 1) and 4 µg/20 µl ($\chi^2 = 17.78$; df = 1; $P < 0.0001$) (fig. 9D). The insect was attracted to nonanal at 3 µg/20 µl ($\chi^2 = 5.38$; df = 1), 6 µg/20 µl ($\chi^2 = 10$; df = 1) and 12 µg/20 µl ($\chi^2 = 27.78$; df = 1) (fig. 9E).

Discussion

This study indicated presence of 11 alcohols, 3 aldehydes, 2 ketones and terpenoids among the 23 VOCs identified in the four varieties (Bio L 212 Ratan, Nirmal B-1, WBK-14-7 and WBK-13-1) of khesari seeds. Nonanal was the major compound followed by farnesyl acetone in Bio L 212 Ratan and WBK-13-1 khesari seeds; whereas farnesyl acetone was predominant followed by nonanal in Nirmal B-1 and WBK-14-7 khesari seeds. Thirteen VOCs were detected from uninfested cowpea seeds, and nonanol was the major compound

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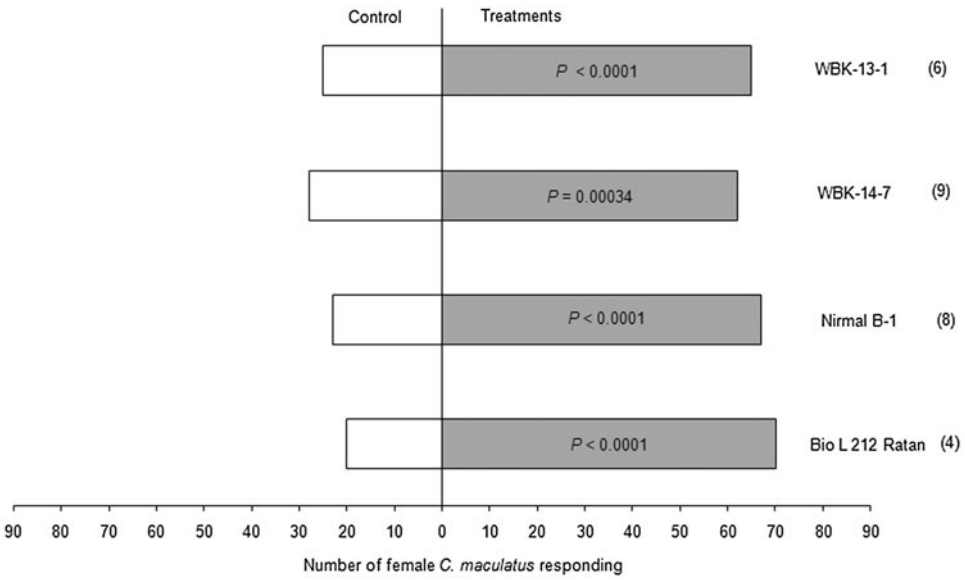

Fig. 5. Female *C. maculatus* responses to four varieties of khesari seeds volatiles: Bio L 212 Ratan or Nirmal B-1 or WBK-14-7 or WBK-13-1 vs. solvent (CH_2Cl_2) control in Y-tube olfactometer bioassay. Numbers in brackets are the number of insects that did not respond to either treatment.

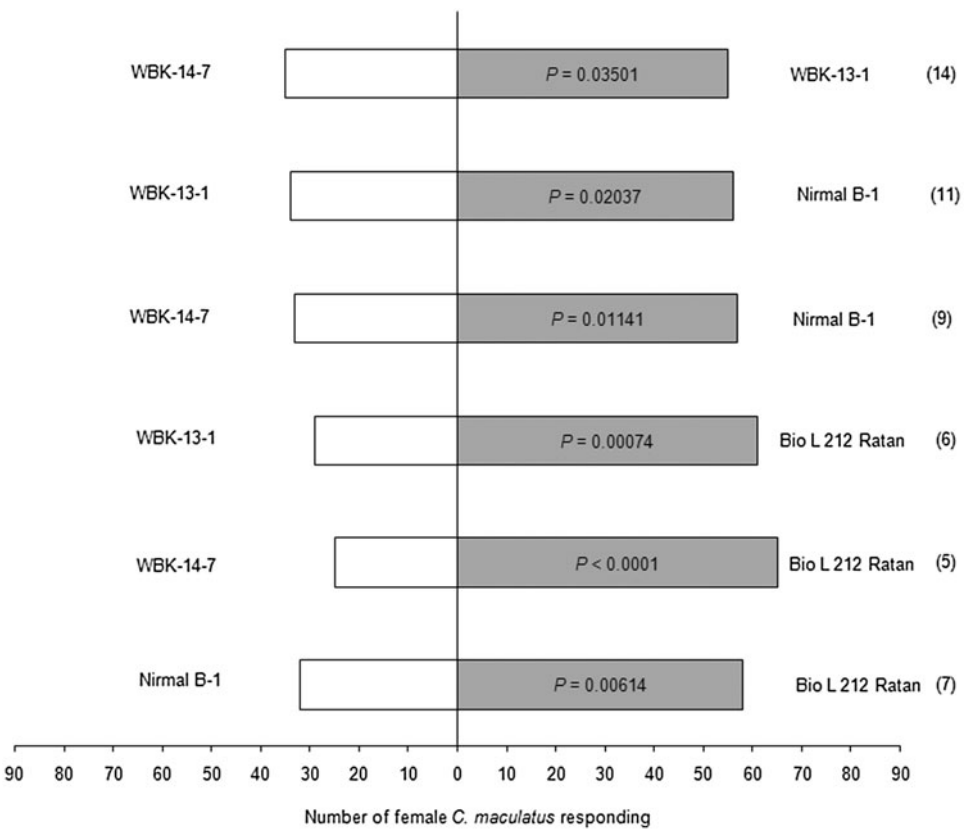

Fig. 6. Female *C. maculatus* responses to four varieties of khesari seeds volatiles tested against each other: Bio L 212 Ratan vs. Nirmal B-1, Bio L 212 Ratan vs. WBK-14-7, Bio L 212 Ratan vs. WBK-13-1, Nirmal B-1 vs. WBK-14-7, Nirmal B-1 vs. WBK-13-1, and WBK-13-1 vs. WBK-14-7 in Y-tube olfactometer bioassay. Numbers in brackets are the number of insects that did not respond to either treatment.

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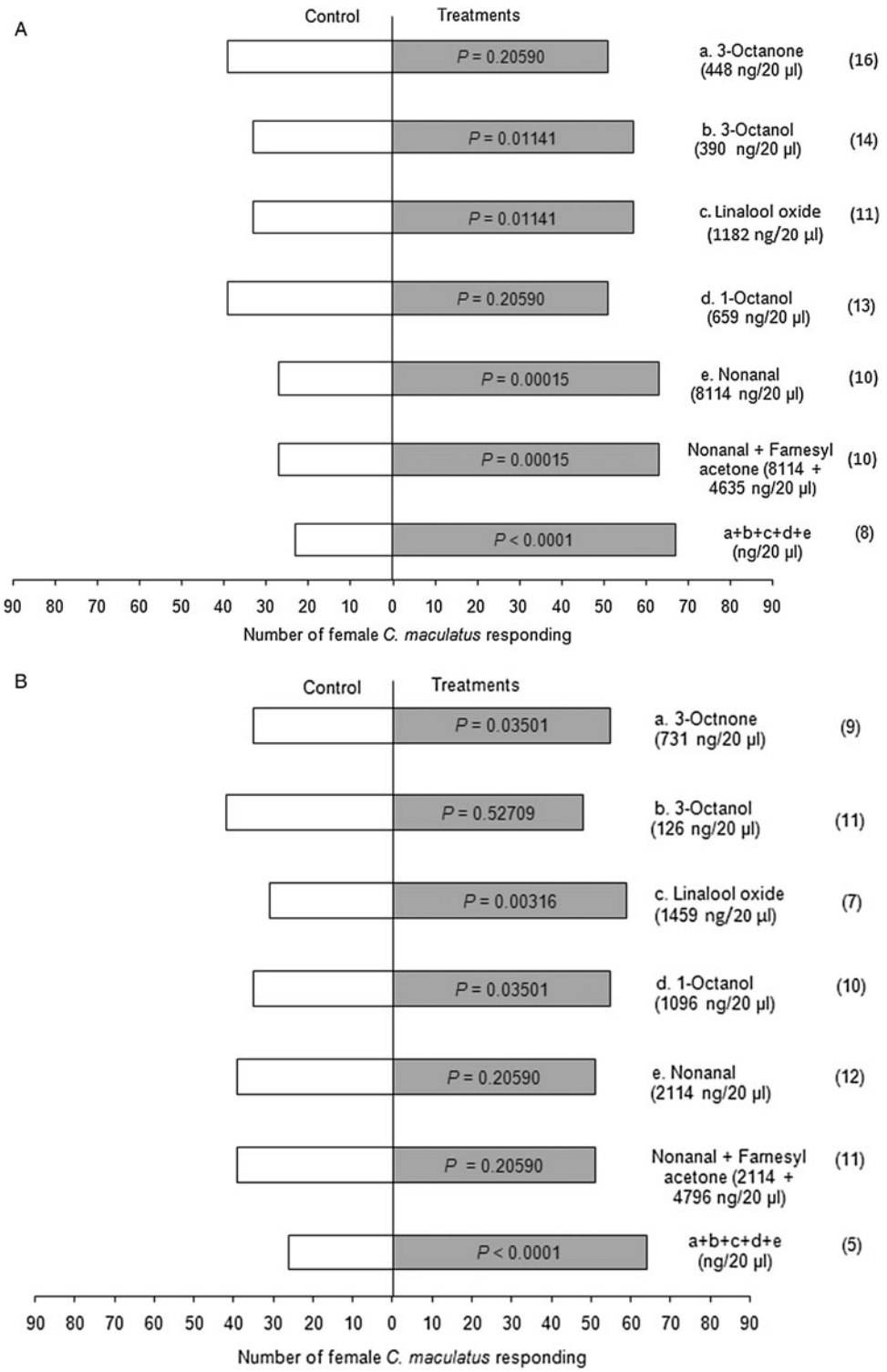

Fig. 7. See the following page for legend.

followed by benzaldehyde, decanal, tridecane and hexanal in uninfested cowpea seeds (Babu *et al.*, 2003).

The olfactometric bioassay results clearly revealed that *C. maculatus* could discriminate between the whole volatile blends released from each variety of khesari seeds against control solvent, and the insect preferentially respond to Bio L 212 Ratan seeds among the four varieties of seeds due to presence of higher amounts of 3-octanol and nonanal than other three

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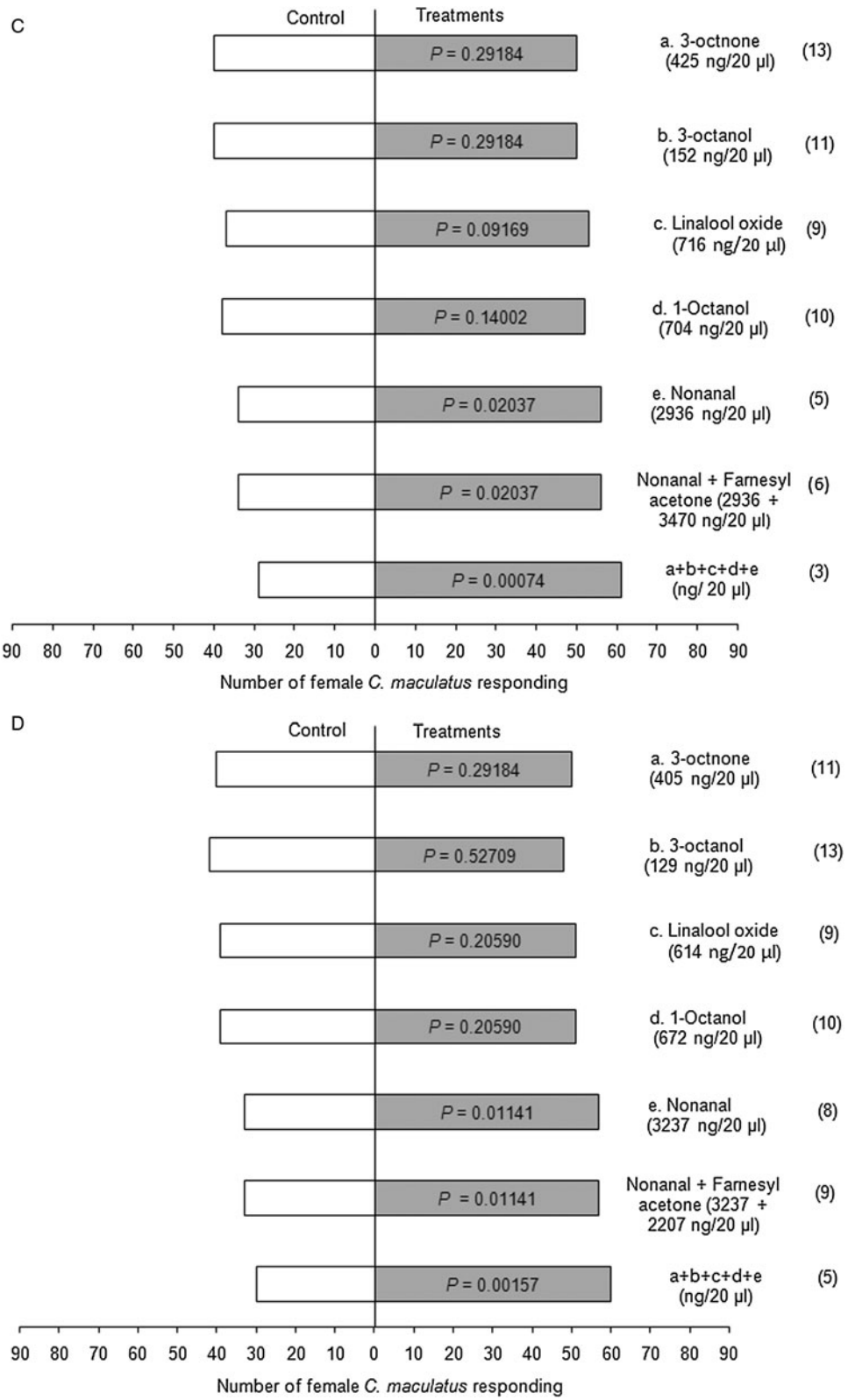

Fig. 7. Female *C. maculatus* responses to individual synthetic compounds and their combinations equivalent to the proportions of: Bio L 212 Ratan (a) or Nirmal B-1 (b) or WBK-14-7 (c) or WBK-13-1 khesari seeds (d) vs. solvent (CH_2Cl_2) control in Y-tube olfactometer bioassay. Numbers in brackets are the number of insects that did not respond to either treatment.

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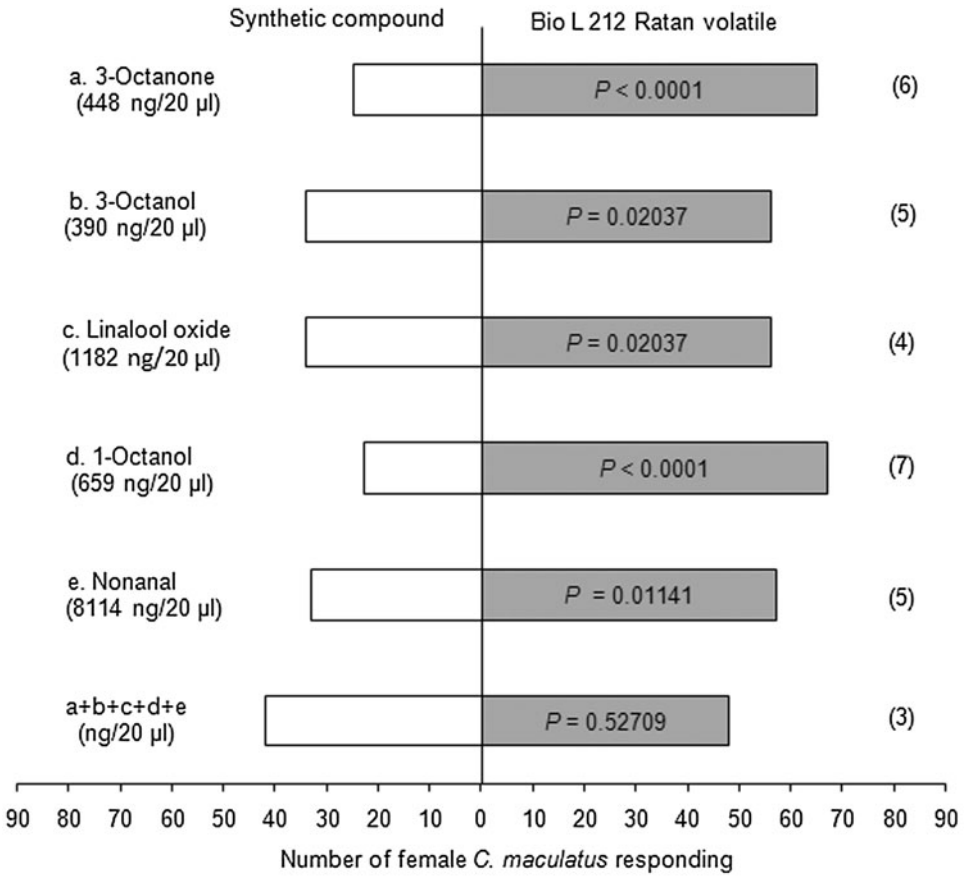

Fig. 8. Female *C. maculatus* response to volatiles extracted from Bio L 212 Ratan khesari seeds vs. individual synthetic compound or a combination of synthetic compounds in the proportions present in Bio L 212 Ratan khesari seeds.

varieties of khesari seeds (as seen in table 1). Insects employ between the ranges of 3–10 compounds as host location cue (Bruce & Pickett, 2011). *Callosobruchus maculatus* showed attraction to 3-octanol, linalool oxide and nonanal at the minimal amounts of 390, 1182 and 2936 ng/20 µl in initial bioassays at the proportions detected in the khesari seeds, respectively; whereas the insect did not produce any positive and negative responses to 3-octanone and 1-octanol at 405 and 659 ng/20 µl, respectively. However, the insect showed attraction to 3-octanone and 1-octanol at the minimal amounts of 1000 and 2000 ng/20 µl in dose response bioassays, respectively. Hence, the ratio of volatiles released by khesari seeds becomes vital components, which act as olfactory cue for *C. maculatus* (Bruce *et al*., 2005; Bruce & Pickett, 2011). Except linalool oxide, the above mentioned four volatile compounds have been reported to affect behavioral responses in a number of stored grain insect pests (Pierce *et al*., 1991; Sinha, 1991; Collins *et al*., 2007). However, the polyphagous beetle, *Popillia japonica* Newman showed attraction to linalool oxide with other compounds which are emitted from *Malus domestica* (Rosaceae) leaves (Loughrin *et al*., 1995).

The VOCs from four varieties of khesari seeds indicated emissions of several compounds including benzyl alcohol and linalool. It is well established that benzyl alcohol and linalool might play an important role in defense such as facilitating attraction of natural enemies to the insect pest (De Moraes *et al*., 1998; Tabata *et al*., 2011). In the present olfactory bioassay result, we also observed no significant attraction of the test insect to synthetic benzyl alcohol and linalool when these compounds were tested in isolation. Farnesyl acetone, which was predominant in Nirmal B-1 and WBK-13-1 khesari seeds, did not indicate any attraction to the beetles. Further, 1-heptanol, 1-octen-3-ol, 1-nonanol, 1-decanol and phytol, were moderately abundant in the khesari seeds (as seen in table 1), did not display attraction of beetles in Y-shaped olfactometer bioassays. However, the attraction to the overall blend of VOCs released by uninfested khesari seeds cannot be ruled out, as insect responses to olfactory foraging cues depend on overall volatile blend rather than on attraction of individual compounds (Riffell *et al*., 2009; Webster *et al*., 2010).

In summary, our findings document that total amount of volatiles were higher in Bio L 212 Ratan followed by Nirmal B-1, WBK-14-7 and WBK-13-1 khesari seeds, and female *C. maculatus* displayed the lowest attraction toward WBK-14-7 and WBK-13-1 khesari seeds. Hence, this study indicates that farmers should be promoted for production of WBK-14-7 and WBK-13-1 khesari seeds, but it remains to be seen whether the food value of the two varieties of seeds are less than Bio L 212 Ratan and Nirmal B-1 khesari seeds. Further, *C. maculatus* were most attractive to a synthetic blend of 448, 390, 1182, 659 and 8114 ng/20 µl of

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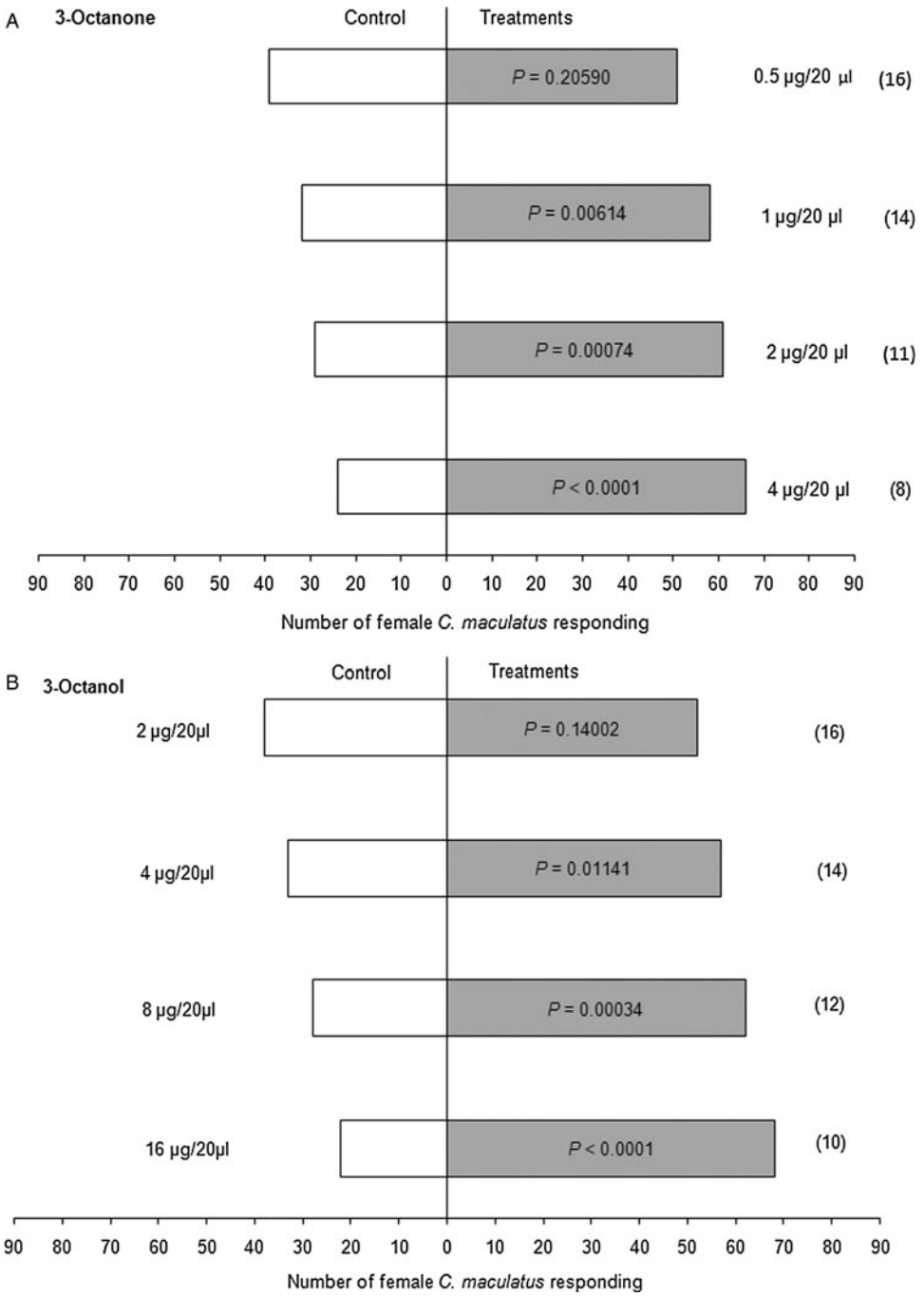

Fig. 9. See the following page for legend.

3-octanone, 3-octanol, linalool oxide, 1-octanol and nonanal, respectively, which might be used for the development of much needed eco-friendly trapping tools for pest management of *C. maculatus*. However, female *C. chinensis* prefer to oviposit uninfested cowpea seeds and egg-carrying seeds over L1- and L4-infested seeds (Ignacimuthu *et al.*, 2000), and feeding by the fourth instar larva of *C. chinensis* within the cowpea seeds is directly and or indirectly responsible for production of volatiles which are clearly repellent to conspecific females (Babu *et al.*, 2003). In a recent study, Arnold *et al.* (2012) reported that inactive-form of *C. maculatus* was more attractive to cowpeas than active-form, and inactive females showed strong attraction over inactive males. They further indicated that young and intermediate aged beetles preferred infested and uninfested cowpeas, respectively (Arnold *et al.*, 2012). Hence, further studies are needed to observe whether feeding by larvae of *C. maculatus* induces repellence of conspecific females, and role of sex and

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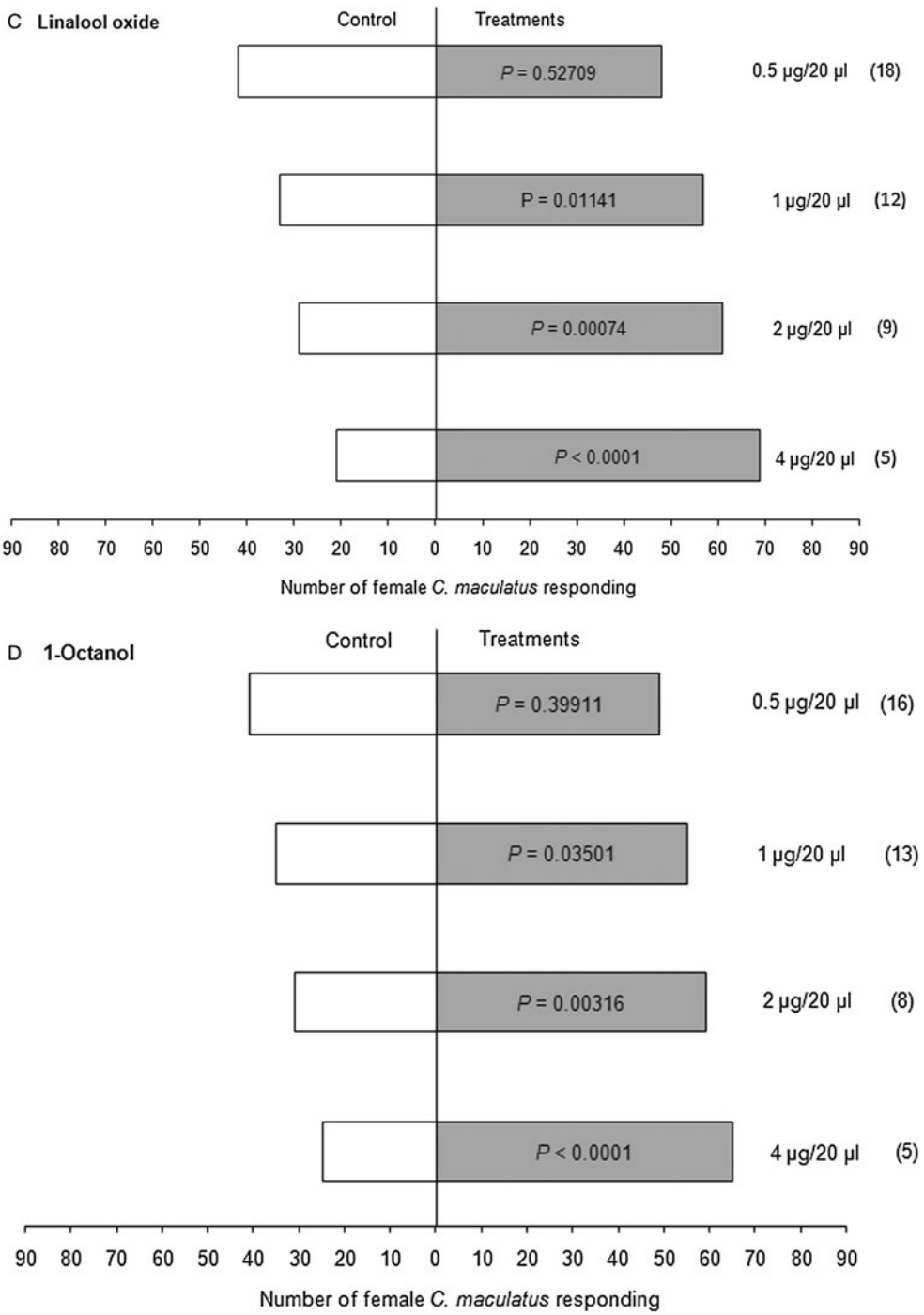

Fig. 9. See the following page for legend.

age in the olfactory behavior of this insect toward uninfested and infested khesari seeds.

Supplementary Material

The supplementary material for this article can be found at http://www.journals.cambridge.org/BER

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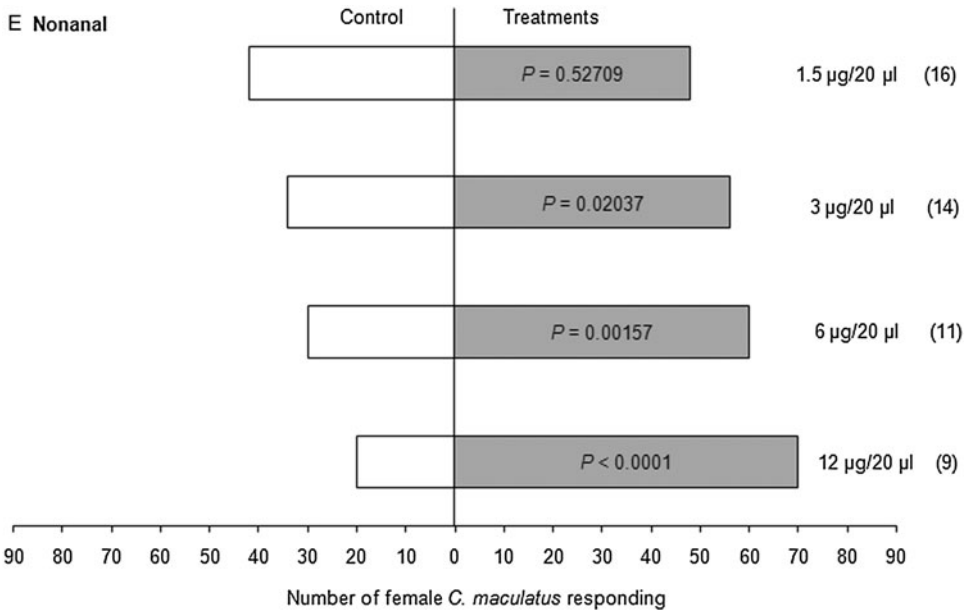

Fig. 9. Female *C. maculatus* response to synthetic volatiles vs. solvent (CH_2Cl_2) control in Y-tube olfactometer bioassay, (a) 3-octanone, (b) 3-octanol, (c) linalool oxide, (d) 1-octanol, (e) nonanal. Numbers in brackets are the number of insects that did not respond to either treatment.

References

Adhikary, P. & Barik, A. (2012) Effect of temperature on biology of *Callosobruchus maculatus* (F.). *Indian Journal of Entomology* **74**, 261–266.

Arnold, S.E.J., Stevenson, P.C. & Belmain, S.R. (2012) Odour-mediated orientation of beetles is influenced by age, sex and morph. *PLoS ONE* **7**(11), e49071. doi: 10.1371/journal.pone.0049071

Babu, A., Hern, A. & Dorn, S. (2003) Sources of semiochemicals mediating host finding in *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Bulletin of Entomological Research* **93**, 187–192.

Bogamuwa, M.M.S., Weerakoon, K.C. & Karunaratne, S.H.P.P. (2002) Insecticide resistance in the bruchid *Callosobruchus maculatus*, a storage pest of legumes. *Ceylon Journal of Science (Biological Science)* **30**, 55–66.

Bruce, T.J.A. & Pickett, J.A. (2011) Perception of plant volatile blends by herbivorous insects – finding the right mix. *Phytochemistry* **72**, 1605–1611.

Bruce, T.J.A., Wadhams, L.J. & Woodcock, C.M. (2005) Insect host location: a volatile situation. *Trends in Plant Science* **10**, 269–274.

Collins, L.E., Bryning, G.P., Wakefield, M.E., Chambers, J. & Cox, P.D. (2007) Progress towards a multi-species lure: identification of components of food volatiles as attractants for three storage beetles. *Journal of Stored Products Research* **43**, 53–63.

De Moraes, C.M., Lewis, W.J., Paré, P.W., Alborn, H.T. & Tumlinson, J.H. (1998) Herbivore-infested plants selectively attract parasitoids. *Nature* **393**, 570–573.

Fox, C.W. (1993) The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Oecologia* **96**, 139–146.

Fox, C.W., Bush, M.L. & Messina, F.J. (2010) Biotypes of the seed beetle *Callosobruchus maculatus* have differing effects on the germination and growth of their legume hosts. *Agricultural and Forest Entomology* **12**, 353–362.

Gaur, V. & Maloo, S.R. (2011) Genetic divergence in grass pea (*Lathyrus sativus*). *Indian Journal of Agricultural Science* **80**, 172–173.

Girma, D. & Korbu, L. (2012) Genetic improvement of grass pea (*Lathyrus sativus*) in Ethiopoia: an unfulfilled promise. *Plant Breeding* **131**, 231–236.

Howe, R.W. & Currie, J.E. (1964) Some laboratory observations on the rates of development, mortality, and oviposition of several species of Bruchidae breeding in stored pulses. *Bulletin of Entomological Research* **55**, 437–477.

Ignacimuthu, S., Wäckers, F.L. & Dorn, S. (2000) The role of chemical cues in host finding and acceptance by *Callosobruchus chinensis*. *Entomologia Experimentalis et Applicata* **96**, 213–219.

Loughrin, J.H., Potter, D.A. & Hamilton-Kemp, T.R. (1995) Volatile compounds induced by herbivory act as aggregation kairomones for the Japanese beetle (*Popillia japonica* Newman). *Journal of Chemical Ecology* **21**, 1457–1467.

Magalhães, D.M., Borges, M., Laumann, R.A., Sujii, E.R., Mayon, P., Caulfield, J.C., Midega, C.A.O., Khan, Z.R., Pickett, J.A., Birkett, M.A. & Blassioli-Moraes, M.C. (2012) Semiochemicals from herbivory induced cotton plants enhance the foraging behaviour of the cotton boll weevil, *Anthonomus grandis*. *Journal of Chemical Ecology* **38**, 1528–1538.

Mbata, G.N. (2004) Cowpea: United States of America. pp. 151–158 *in* Hodges, R.J. (*Ed.*) *Crop Post-harvest: Science and Technology*. Oxford, Blackwell.

Mbata, G.N. & Payton, M.E. (2013) Effect of monoterpenoids on oviposition and mortality of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) under hermetic conditions. *Journal of Stored Products Research* **53**, 43–47.

Messina, F.J. (1991) Life-history variation in a seed beetle: adult egg-laying vs. larval competitive ability. *Oecologia* **85**, 447–455.

https://doi.org/10.1017/S000748531400087X Published online by Cambridge University Press

Mukherjee, A., Sarkar, N. & Barik, A. (2013) Alkanes in flower surface waxes of *Momordica cochinchinensis* influence attraction to *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae). *Neotropical Entomology* **42**, 366–371.

Mukherjee, A., Sarkar, N. & Barik, A. (2014) Long-chain free fatty acids from *Momordica cochinchinensis* leaves as attractants to its insect pest, *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae). *Journal of Asia-Pacific Entomology* **17**, 229–234.

Parr, M.J., Tran, B.M.D., Simmonds, M.S.J., Kite, G.C. & Credland, P.F. (1998) Influence of some fatty acids on oviposition by the bruchid beetle, *Callosobruchus maculatus*. *Journal of Chemical Ecology* **24**, 1577–1593.

Pierce, A.M., Pierce, H.D. Jr., Borden, J.H. & Oehlschlager, A.C. (1991) Fungal volatiles: semiochemicals for stored-product beetles (Coleoptera: Cucujidae). *Journal of Chemical Ecology* **17**, 581–597.

Riffell, J.A., Lie, H., Christensen, T.A. & Hildebrand, J.G. (2009) Characterization and coding of behaviourally significant odor mixtures. *Current Biology* **19**, 335–340.

Roy, N., Laskar, S. & Barik, A. (2012) The attractiveness of odorous esterified fatty acids to the potential biocontrol agent, *Altica cyanea*. *Journal of Asia-Pacific Entomology* **15**, 277–282.

Sarkar, N. & Barik, A. (2014) Alkanes from bitter gourd as allelochemicals in olfactory responses of *Epilachna dodecastigma* (Wied.). *Allelopathy Journal* **33**, 43–51.

Sarkar, N., Mukherjee, A. & Barik, A. (2013*a*) Long-chain alkanes: allelochemicals for host location by the insect pest, *Epilachna dodecastigma* (Coleoptera: Coccinellidae). *Applied Entomology and Zoology* **48**, 171–179.

Sarkar, N., Mukherjee, A. & Barik, A. (2013*b*) Olfactory responses of *Epilachna dodecastigma* (Coleoptera: Coccinellidae) to long-chain fatty acids from *Momordica charantia* leaves. *Arthropod-Plant Interactions* **7**, 339–348.

Sarkar, N., Mukherjee, A. & Barik, A. (2014) Attraction of *Epilachna dodecastigma* (Coleoptera: Coccinellidae) to *Momordica charantia* (Cucurbitaceae) leaf volatiles. *Canadian Entomologist*. doi: 10.4039/tce.2014.37.

Schoonhoven, L.M., Van Loon, J.J.A. & Dicke, M. (2005) *Insect–Plant Biology*. Oxford, Oxford University Press.

Sinha, R.N. (1991) Role of odor volatiles in the stored-grain ecosystem. pp 3–14 *in* Fleurat-Lessard, F. & Ducom, P. (*Eds*) *Proceedings of the 5th International Working Conference on Stored-Product Protection, 9–14 September 1990*, Bordeaux, France.

Tabata, J., De Moraes, C.M. & Mescher, M.C. (2011) Olfactory cues from plants infected by powdery mildew guide foraging by a mycophagous ladybird beetle. *PLoS ONE* **6**, e23799, doi: 10.1371/journal.pone.0023799.

United Nations Environment Programme (1998) Montreal Protocol on Substances that Deplete the Ozone Layer, 1998: Assessment of Alternatives to Methyl Bromide. Methyl Bromide Alternatives Committee, Nairobi, Kenya.

Utida, S. (1967) Damped oscillations of population density at equilibrium. *Researches on Population Ecology* **9**, 1–9.

Utida, S. (1972) Density dependent polymorphism in the adult of *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Journal of Stored Products Research* **8**, 111–125.

Webster, B., Bruce, T., Pickett, J. & Hardie, J. (2010) Volatiles functioning as host cues in a blend become nonhost cues when presented alone to the black bean aphid. *Animal Behaviour* **79**, 451–457.