Host Odorants Guide Host Finding Behaviour in Coconut Caterpillar (*Opisina arenosella* Walker; Lepidoptera: Oecophoridae): A New Strategy for Green Pest Management

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Abstract

Coconut black headed caterpillar, *Opisina arenosella* Walker (Lepidoptera: Oecophoridae) is of serious concern. Owing to its restricted feeding habit it is reasonable to expect *O. arenosella* may use specific host volatiles to find its oviposition and larval feeding sites. Hence, identifying the host volatile cues will aid to develop a robust pest management. Behaviourally active host volatiles can be used for pest monitoring and mass trapping purposes offers a clean and green pest management method.The electrophysiological and behavioural assay was conducted to determine the role of host volatiles for host searching behavior of *O. arenosella*. Over 50 volatile organic compounds were identified from damaged and undamaged coconut leaf. Among them six volatiles were electrophysiologically active viz., linalool, hexanal, hexanol, acetophenone, nonanal and limonene. Behavioural assay revealed that, moths irrespective of sex responded to linalool. Females were more receptive to linalool, acetophenone and limonene for oviposition. Taxis responses of male moth revealed that they were significantly attractive for linalool and acetophenone in wind tunnel. The responses of male and female to blends of linalool and sex pheromone (1:1) (BHC 20) and acetophenone (2:1) (BHC 24) were significantly higher than sex pheromone blend (BHC 1) in EAG and wind tunnel assays. Owing to mass trapping of male and female, BHC 20 and BHC 24 can be recommended to management of *O. arenosella* after field evaluation.

Keywords: *Coconut black headed caterpillar, Electrophysiology, Host finding behavior, Plant semiochemicals, Oviposition preference*

Introduction

Coconut (*Cocos nucifera* Linn., Palmaceae) is a major plantation crop widely cultivated in tropical areas. Sri Lanka is the fifth largest coconut producer in the world with an area of 0.4 million ha and production of 3000 million nuts per year. (Coconut stat, 2014).

All parts of the coconut palm are attacked by hundreds of pests round the year *viz.*, fronds, nuts, inflorescence, stem and roots. Among

the pests that attack the foliage, coconut black headed caterpillar, *Opisina arenosella* Walker (Lepidoptera: Oecophoridae) is of serious concern. *O. arenosella* is a multivoltine tropical moth having discrete generation cycles with 5 or 6 generations per year (Muralimohan and Srinivasa, 2009). Pest was first reported from Batticoloa in Sri Lanka in 1848 (Green, 1906a and 1906b; Perera, 1987; Perera *et al.*, 1989). *O. arenosella* larvae damage coconut palms in Sri Lanka, India, Bangladesh, Myanmar, Indonesia, Thailand and China (Perera *et al.*, 1989; Srinivasa and Muralimohan, 2009; Bao-qian *et al.*, 2013; Li *et al.*, 2014). The female moth lays eggs on underside of the coconut leaflets; the larvae feed on parenchymal tissues of coconut leaflets, constructing galleries using faecal matter and silk (Perera, 1987; Ramkumar, 2002). Gregarious feeding by larvae in the leaflets leaving only the upper epidermis gives a scorched appearance to fronds. From a distance, infested orchards, show a burnt-up appearance with drastically reduced green tissue of the canopy and progressing upwards. Severe infestation leads to reduced nut production, increased premature nut fall and growth retardation (Lever, 1969; Mohandas, 1992). Larval damage on fronds causes >50 % crop loss in subsequent year after severe outbreak and it takes four subsequent years for the palms to regain their normal yield (Chandrikamohan *et al.*, 2010). Several chemical, biological and cultural methods of control have been developed in the past but practical difficulties have put forward an urgent need to explore an alternative eco-friendly practical approach.

Exploiting the ethology of the pest is an effective method in pest management. The volatile organic compounds form host serve as a tool to attract or repel the pest from its host. Owing to coconut black headed caterpillar's restricted feeding habit, it is reasonable to expect *O. arenosella* may use specific host odours (host volatiles/ kairomones) to find its oviposition and larval feeding sites. Exploiting the behaviourally active host kairomone as a potential tool for pest monitoring and mass trapping purposes offers a clean and green pest management method. The present investigation on the role of host volatile for host selection behaviour of *O. arenosella* was conducted to identify behaviourally active potential volatile compounds and their use for caterpillar management.

Materials and Methods

Plant volatile collection by solvent extraction method

Solvent extraction method was followed to volatile collection from coconut samples of both larval damage and undamaged leaf. Three organic solvents were used for extraction *vis.* Hexane, Acetone and Dicloromethane (DCM) HPLC grade. Eighty gram of small cut pieces (3mm) of coconut leaflets were put into the 500 ml, Borosil® reagent bottle and 100 ml of solvent was poured into the bottle. The set-up was kept overnight soaking. Soaked solvent was gently decanted into volatile collection vial (Borosil® 15ml). The collected volatile was kept in the deep freezer (under -20 °C) until used for analysis and bioassays. The 1000 µl of collected volatile was condensed to 500 µl by passing gentle stream of ultra-high pure nitrogen. Sample vials with extracts were stored in -20 °C until analysis using Gas chromatography coupled mass spectrometry (GC-MS).

Chemical identification of volatiles

Collected volatile samples were chemically identified using Gas Chromatography coupled Mass Spectrometry (GC-MS) at the Entomology

Division, CPCRI, Kasaragod. GC-MS analyses were performed by a 7890A Gas Chromatograph system (GC)(Agilent technologies) interfaced with a 5975C Mass Spectrometer Detector (MSD) (Agilent technologies) with triple axis detector (electron impact ionization, 70 eV) through a HP-5MS phenyl silox capillary column (inner diameter 30 m × 0.25 mm) (J & W Scientific, Folsom, California). The temperature of column and oven were maintained at 40 °C for 1 min. and then increased @ 20 °C/min to 280 °C and held at 300 °C for 10 min. The injector and column temperatures were 250 °C. The total run was for 23 min. The mass spectrometry data library was NIST 08 and ms search 2.0 software was used in the analyses. Internal standard Bromodecane 100 ng was used. One micro liter of concentrated volatile was injected in to the machine using Chromatographic (Hamilton) syringe.

Electroantennogram (EAG)

Electroantennogram (EAG) studies were carried out to determine the electrophysiological responses for the identified individual compounds. Pure synthetic (>99% purity) volatile compounds brought from Sigma were used for the study. EAG responses of adults female and male were made using a commercially available electroantennographic system (Syntech, Hilversum, The Netherlands) consisting of a dual electrode probe for antenna fixation, a CS-05 stimulus controller and an IDAC 232 box for data acquisition system. The antenna was removed from the 1-2 days old moth using fine surgical bent scissors. The detached antenna was fixed with the tip of one of the electrode and scape was fixed to the other electrode. The antenna was fixed between the two electrodes using Spectra 360® conductive gel (Parker, Orange, New Jersey). The antenna was flushed continuously with stream of activated charcoal filtered air.

The volatile solutions (5 µl) were applied on filter paper strips (Advantec 5C, 110 mm) Japan of 3 cm length and 5mm diameter and were placed into the micro tip pipettes (Tarson 100–1000 µl) and was connected to stimulus controller by trygon rubber tube. After 10 seconds the solvent was blown out with first puff. Another 60 seconds later, the stimulus was puffed on to the antenna by injecting the vapor phase of the micro tip pipette 15 mm upstream from the antennae in the continuous air stream (pulse time 0.5 seconds, continuous flow 25 ml/s, pulse flow 21 ml/s). Air was puffed as standard after every batch. Antennal response to aliquots and host volatiles was recorded from both mated and unmated female moths with 5 replications. Responses were expressed as numbers of action potentials, sorted according to shape and amplitude, emitted during 1 sec after the onset of the stimulation. The mean antennal response data were compared using One way ANOVA followed by Tukey post hoc test using IBM SPSS version 21.

Y- tube olfactometer studies

Behavioural studies were conducted to determine the behavioural responses of EAG active volatiles. Tests were performed using a Y-tube glass olfactometer (inner diameter 40 mm). The vertical segment was 160 mm long and the arms were 150 mm long, spaced at an angle of 120°. During the test, odours (filter paper with dose of 2 µl volatiles were placed inside 1000 µl disposable micropipette tips) were placed at the end of one arm of the Y-tube and (as same pipette tip inside filter paper without odour) was placed in the other arm. This sequence was interchanged randomly in subsequent replicates. The suction pump was fitted to the vertical segment (stem of Y-tube) outlet end to gently suck the odours from the arms. An adult insect container with five test moths was inserted from the end of the vertical

segment. The whole system was kept under red light. Once, moths were started to activate, the suction pump start and odour source fixed to the arm of olfactometer. The number of moths that chose each arm was recorded after 5 min. The Y-tube was replaced with a clean one for each odour source tested. Each moth was exposed to an odour only once. The moths that did not respond were not recorded. Records of fifty individuals were maintained for each odour. Percentage responses were compared by Chi-square test as assuming the equal probability (50:50) for both arms using IBM SPSS version 21.

Oviposition preference assay

Oviposition preference assay was conducted to make sure the *O. arenosella* female used host volatile for oviposition site selection. The dual choice oviposition chamber was prepared using transparent polystyrene cylinders (0.45 m length and 0.10 m diameter). Both sides of the cylinder were covered with two layer of muslin cloth to facilitate aeration, oviposition substrate and odour placement. Cylinder was provided with moisture and honey solution (10%) by placing the soaked folded tissue paper at middle of the cylinder. Assay was conducted using identified synthetic electrophysiologically active volatile compounds *i.e.*, 1-Hexanol, Acetophenone, Linalool, Limonene, Hexanal and Nonanal. Odour source was placed on the cloth in one side and other side was used as control (or blank). Each compound, 1 µl volume with concentration 10^{-4} were used separately and compound diluted in DCM. Odour source was renewed daily until female die. Number of eggs laid on each side was counted and removed daily. Numbers of eggs were compared by paired t test using IBM SPSS version 21.

Formulation of volatile blends

Two volatile blends (BHC 20 and BHC 24) were formulated based on the series of experimental results (Kumara, 2015). BHC 20 consists linalool and sex pheromone (1:1) while BHC 24 a mixture of acetophenone and sex pheromone (1:2). Volatile compound was diluted in hexane (HPLC grade) and made a 10 mg/ml stock solution. Stock solution was diluted ten times in hexane and made the 1 mg/ml working solution. In each assay volatiles were compared with hexane as the solvent control and female sex pheromone blend as the positive control. Twenty microliters of working solution was used for EAG bio assays. Both female and male were done in each set and study repeated minimum 5 per sex. Responses were compared by One way ANOVA followed by Tukey post hoc test using IBM SPSS version 21.

Wind tunnel bioassay

The blends which having best electrophysiological response from EAG were further tested to determine the behavioural effects in a wind-tunnel (1.2 × 0.3 × 0.3 m). Behavioural assays were conducted at Insect Behaviour Testing Laboratory, BCRL at 24 ± 1 °C and 65 % ± 5 RH. The average velocity of airflow within the wind-tunnel was 25 cm s^{-1} at lure source point and diffused light intensity of 5 lux was used in these studies. The airflow was produced by an electrical fan purified by a 0.05 m layer of activated charcoal filter. Male response was tested between 4th h of scotophase. One day old 10 moths were placed in a perforated acrylic rectangular cage (5 × 5 × 15 cm) kept at opposite end of the airflow. Moths were allowed to acclimatize to the tunnel condition for at least one hour prior to testing. Exactly 20 µl of blend aliquot was impregnated in the piece of the filter paper (5 × 0.5 cm; Whatman No. 1) and the filter

paper was hung 0.15 m high from the floor of the wind tunnel. The blank (hexane) treatment was tested first to check that the wind-tunnel was not contaminated. Moths were allowed to naturally leave the cage just after setting a stimulus source at the upwind end. The responses of both males and females were assays separately. The responses of moths to the sources were scored different behavioural categories *i.e.* Took flight, Up wind flight, Source approach and Source contact. Observation was recorded in 2 hrs and was repeated with new set of moths. The number of moth responses were compared by chi-square test using IBM SPSS version 21.

Results and Discussion

Identification of host plant volatile

A total of 50 volatile organic compounds were identified from undamaged coconut leaves, and larval damaged leaflets (Table 01 and Figure 1). Based on the literature search those that cause physiological response in other insects were selected for EAG studies the compounds include acetophenone, 1-hexanol, 1-hexanal, nonanal, linalool, limonene, β- myrcene, 3 carene, β-pinene, trans-2- hexen-1-ol and nonanol. All compounds selected in the current study were terpenoids and green leaf volatiles (GLV's) which is in corroboration with other studies who also selected the same for semiochemical studies on host searching behaviour of herbivore insects (Fraser *et al.*, 2003; Webster, *et al.* 2008; Webster *et al.* 2010; Ukeh *et al.* 2012; Lu *et al.* 2012).

Table 1. Volatile profile of undamaged coconut leaf (West Coast Tall) by solvent extraction method

Compound	Relative abundance (%)
Emylcamate	0.13
Limonene	0.509
Trans-3-Penten-2-ol	0.096
1-Hexen-1-ol	0.158
(Z)-Ethylbenzene	0.167
p-Xylene	0.505
Hexylene Glycol	0.042
2-Hexen-4-olide	0.113
Benzaldehyde	0.043
Acetophenone	3.013
E-2-Hexenyl benzoate	0.967
Phenylethyl Alcohol	0.658
Tridemorph	1.198
Ethanol, 2-phenoxy	5.833
Linalool	4.979
Isopropyl Myristate	2.004
Hexahydrofarnesyl acetone	3.007
Phytol	3.653
Heptacosane	1.209
Benzyl alcohol	1.65

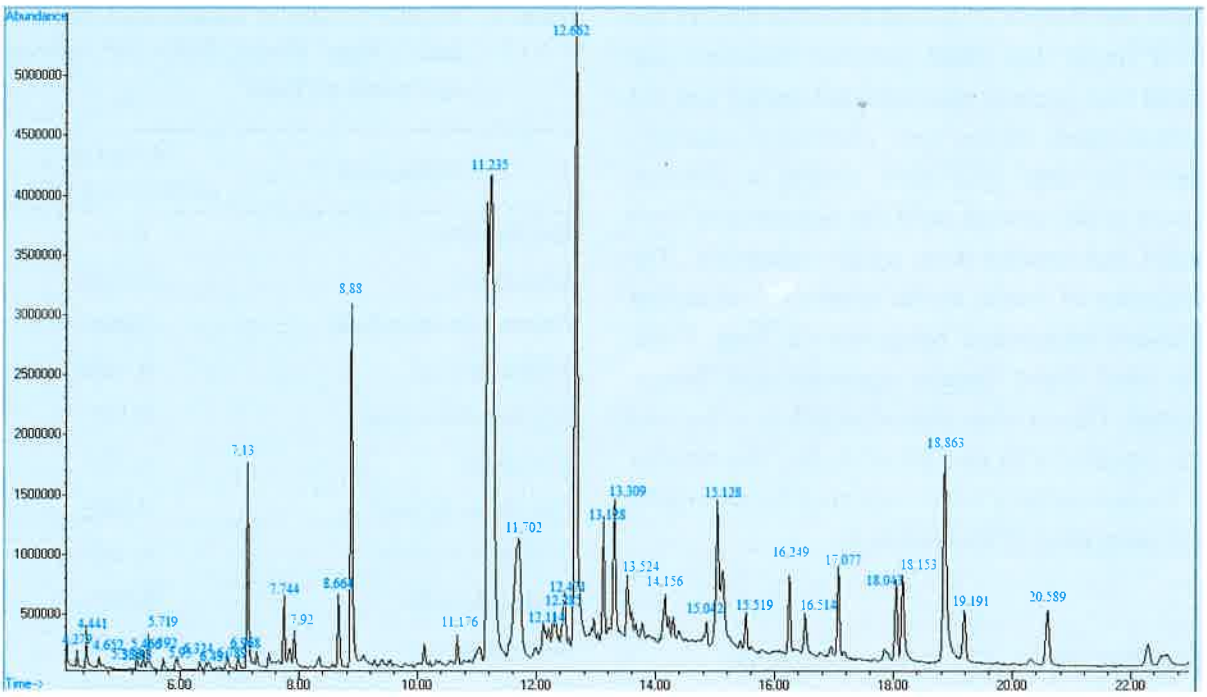

Figure 1. Representative GC-MS traces of undamaged coconut leaf volatiles (WCT variety) by solvent (DCM) extraction method

EAG bioassay of male moth responses revealed that there was a significant difference among the tested volatiles ($F_{7, 32} = 35.240$; $p = 0.0001$). Acetophenone and hexanol evoked the highest response and these two were on par. Linalool and limonene evoked the second higher response and are significantly lower than acetophenone and hexanol. Blank air evoked the lowest response (Table 2).

EAG bioassays

EAG bioassay of female responses were significantly different among the tested compounds while hexanol and linalool were evoked significantly higher responses than other compounds and were followed by acetophenone and nonanal (Table 3).

Table 2. EAG responses of *O. arenosella* male to selected plant volatiles

Treatment	EAG Response (mV) (Mean ± SE)
Air	0.01 ± 0.004^{d}
Linalool	0.11 ± 0.002^{b}
Nonanal	0.04 ± 0.002^{cd}
Limonene	0.11 ± 0.008^{b}
Hexanal	0.07 ± 0.005^{c}
Hexanol	0.15 ± 0.009^{a}
Coconut leaf	0.07 ± 0.017^{c}
Acetophenone	0.16 ± 0.008^{a}

Different letters with the figures are significantly different at 5 % (Tukey post hoc test) (N= 5)

Table 3. EAG responses of *O. arenosella* female to select plant volatiles

Treatment	EAG Response (mV) (Mean ± SE)
Air	0.019 ± 0.004^{bc}
Trans-2- hexen-1-ol	0.09 ± 0.02^{abc}
β-pinene	0.06 ± 0.02^{abc}
3-caren	0.01 ± 0.002^{c}
β- myrcene	0.10 ± 0.003^{abc}
Nonanal	0.11 ± 0.001^{abc}
Linalool	0.13 ± 0.03^{a}
Nonanol	0.11 ± 0.001^{ab}
Limonene	0.05 ± 0.03^{abc}
Hexanal	0.02 ± 0.004^{bc}
Hexanol	0.13 ± 0.005^{a}
Coconut leaf	0.07 ± 0.02^{abc}
Acetophenone	0.12 ± 0.02^{ab}

Different letters with the figures are significantly different at 5 % (Tukey post hoc test) (N = 5)

Among the tested volatiles, acetophenone, linalool, limonene, hexanal, hexanol and nonanal were selected as electrophysiologically active compounds and subjected to bioassays to determine the behavioural responses (Y tube olfactometer studies).

Behavioural assays

Dual choice Y-tube olfactometer study indicated that, significantly higher number of virgin females were attracted towards linalool ($\chi2 = 6.897$, df = 1, p = 0.009) and limonene ($\chi2 = 4.787$, df = 1, p = 0.029) compared to control. Though higher number of females were attracted towards acetophenone, it was not statistically significant compared to control (Table 4).

The behavioural responses of mated females towards tested volatile compounds and control indicated that, significantly higher percentage of mated females were attracted to linalool and acetophenone compared to control ($\chi2 = 8.1$, df = 1, p = 0.004) ($\chi2 = 5.565$, df = 1, p = 0.018). However, the responses towards limonene and hexanol were higher than the control but not significantly different. Whereas mated females were more attracted towards the blank arm of the olfactometer while testing the hexanal (Table 5).

Table 4. Behavioural response of *O. arenosella* virgin females to plant volatiles

Treatment	Number of response		Chi square value	‡Responded individual (N)	p	
	Source	control				
Hexanal	19	25	0.818	44	0.366	NS
Hexanol	26	20	0.783	46	0.376	NS
Acetophenone	29	17	3.13	46	0.077	NS
Limonene	31	16	4.787	47	0.029	*
Linalool	38	11	6.897	49	0.009	**
Blank	21	17	0.027	38	0.869	NS

* Percentage response between source and control is significantly different at $p<0.05$

** Significant difference at $p<0.01$; NS - non-significant different. ‡ n = 50

Table 5. Behavioural response of *O. arenosella* mated` females to plant volatiles

Treatment	Number of response		Chi square	‡Responded individual (N)	p	
	source	control				
Hexanal	12	22	2.941	34	0.086	NS
Hexanol	20	16	1.000	36	0.317	NS
Acetophenone vs Blank	31	15	5.565	46	0.018	*
Limonene vs Blank	22	15	1.324	37	0.250	NS
Linalool vs Blank	29	11	8.100	40	0.004	**
Blank vs Blank	13	12	0.486	25	0.841	NS

* Percentage response between source and control is significantly different at $p<0.05$

** Significant difference at $p<0.01$; NS- non-significant different. ‡ $n = 50$

The male behavioural response results indicated that, significantly higher number of males were attracted to linalool than control ($\chi 2 = 3.846$, df = 1, $p = 0.05$). Though, male moth attraction towards the female sex pheromone component (standard pheromone of *O. arenosella*) was higher than the control it was not significantly different. The male responses of other tested volatiles were not significantly different over control. However, the male moth responses to acetophenone, limonene and hexanol was higher than the control (Table 6).

Table 6. Behavioural response of *O. arenosella* males to plant volatiles

Treatment	Number of response		Chi square	‡Responded individual (N)	p	
	source	control				
Hexanal	12	14	0.154	26	0.695	NS
Hexanol	11	13	0.167	24	0.683	NS
Acetophenone	15	9	1.500	24	0.221	NS
Limonene	14	9	1.087	23	0.297	NS
Linalool	18	8	3.846	26	0.050	*
Blank	13	11	0.167	24	0.683	NS
BHC pheromone	22	11	3.667	33	0.056	NS

* Percentage response between source and control is significantly different at $p<0.05$

NS - non-significant difference. ‡ $n = 50$

Host searching behaviour of female and male moths indicated that, both were attracted to linalool. The linalool is common volatile organic compound in plants. This may be the reason as both the sexes are in the same niches and utilize common compounds used for host selection, oviposition site selection and mate finding. In some insects like *Hyalesthes obsoletus* Signoret (Hemiptera, Cixiidae) showed sex-specific differences between behavioural responses to

plant VOCs due to different ecological niches that male and female occupy (Sun *et al.*, 2014). Females of both virgin and mated were showed a repellent response to hexanal. However males were not responded to hexanal and other volatiles tested significantly. Responsiveness of linalool to lepidoptera gave attraction, repellence and nonresponses. Sun *et al.* (2014) observed that in their Y tube olfactometer studies both virgin female and male of tea looper caterpillar, *Ectropis obliqua* Prout (Lepideptera: Geometridae), were showed the repellence to linalool however mated females were not responded.

Dual choice oviposition preference assay for synthetic host volatiles

Oviposition preference assay results indicated that, the female laid significantly higher number of eggs on volatile treated side than control side of the oviposition chamber treated with linalool, acetophenone and limonene. However, there was no significant difference in treated with hexanol, hexanal and nonanal over control (Table 7). The highest mean percentage eggs on both acetophenone limonene and linalool treated side of the oviposition chamber revealed that female used these volatiles (terpenoids) for oviposition site selection. In agreement with present study, Bruce *et al.* (2005) and Anfora *et al.* (2009) reported that polyphagous insects rely on the absolute and relative amounts of such common host volatiles during the process of finding oviposition sites while monophagous insects use host specific volatiles for selection of oviposition site. Further, volatiles are identified from host plants by Revadi *et al.* (2015) reported *D. suzukii* mated females are attracted to volatiles emitted from intact fruits. Present study results indicated that the restricted feeding habitat *O. arenosella* used the chemical cues present in both larval frass and coconut leaflet volatiles for ovipositional site selection (Kumara, 2015; Ramkumar, 2002). However, insect species are mostly sensitive not only to individual volatiles but also to the different mixtures of volatile compounds. Therefore, it is essential for testing mixture of compounds against the oviposition site selection behaviour of female moths.

Table 7. Eggs laid by female on the dual choice oviposition assay

Volatile	Number of eggs/ Female (Mean ± SE)		N	t	P
	volatile side	Control			
Acetophenone	215.40 ± 14.29	30.20 ± 6.93	5	11.66	0.0001**
Limonene	188.80 ± 22.32	49.00 ± 9.04	5	5.805	0.002**
Linalool	214.00 ± 24.84	21.00 ± 4.08	5	7.667	0.0001**
Hexanol	106.8 ± 13.58	115.00 ± 13.79	5	0.424	0.683
Hexanal	68.20 ± 14.63	95.60 ± 36.70	5	0.694	0.508
Nonanal	106.80 ± 17.94	88.60 ± 23.17	5	0.621	0.552
Control	110.60 ± 18.41	127.60 ± 17.59	5	0.668	0.523

** Mean number of eggs between volatile side and control was significantly different at 0.01%, (paired samples t- test).

Oviposition discriminate index (ODI) was calculated for each compound based on the female preference against blank (Figure 2). ODI results revealed that there were significant differences among the tested volatiles ($F_{5, 24} = 7.978$, $p = 0.0001$). Linalool had the highest ODI value followed by acetophenone and limonene were significantly different from others while hexanol record the negative ODI. Hexanal and nonanal had the positive ODI but are significantly lower than from remaining (Figure 2).

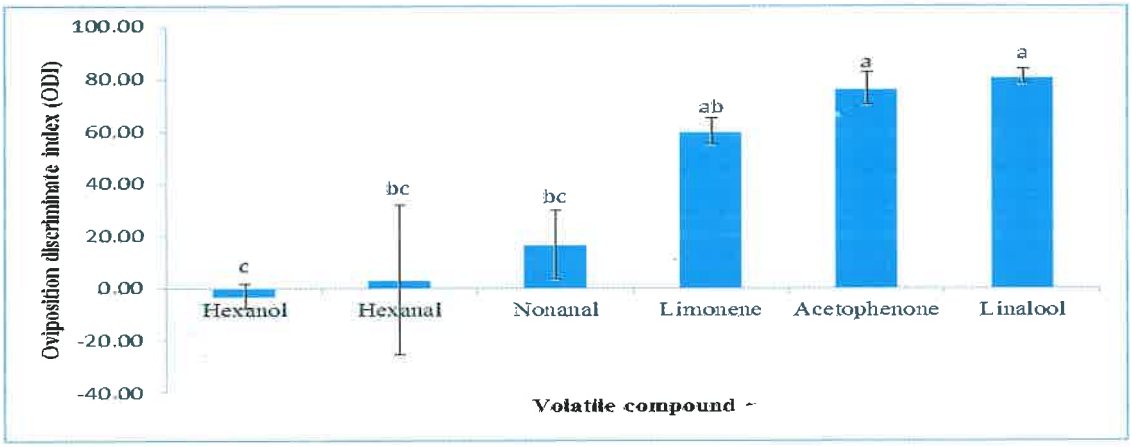

Figure 2. ODIs for *O. arenosella* females to different volatiles
Different letters on the bars are significantly different, Error bars indicates the standard error of means (n = 5) (Maher and Thiery 2006; Tasin et al., 2011)

Oviposition preference study indicated that, *O. arenosella* female was attracted to these compounds individually and as combination. Most plants produce both phenolic compounds and linalool as a result of herbivore damage. Moreover mechanically damaged and herbivore damaged plants produced volatile chemicals due to stress and these volatiles are species specific (Reisenman *et al.*, 2013). Intact plant produces volatiles but at a lower amount than damaged plant. *Manduca sexta* female were repelled and avoid oviposition when larvae damaged tomato plants (*Solanum lycopersicum*) and it was suggested that, damaged plants produced significantly higher quantity of linalool and it repel the oviposition of female *M. sexta* (Reisenman *et al.*, 2010; Reisenman *et al.*, 2013) contradicting the present study. Interestingly our results revealed that *O. arenosella* female was more attractive to linalool, acetophenone and limonene, which are mostly produced in higher quantity due to herbivore and mechanically damaged tissues (Reisenman *et al.*, 2013). The possible reason could be the evolutionary adaptation of *O. arenosella* female to lay eggs on larval frass or damaged leaflet cracks and crevices unlike other insects which avoid laying eggs on these substrates (Rojas, 1999; Almohamad *et al.*, 2010; Anderson *et al.*, 1992; Xu *et al.*, 2006).

Table 8. Taxis response of *O. arenosella* male moths to different blends at 20 µl in wind tunnel

Blend	Replications	TF	UF	SA	SC
BHC 1	20	14	13[a]	5[ab]	0
BHC 20	20	18	14[a]	12[a]	0
BHC 24	20	15	11[a]	8[a]	0
Control (Solvent)	20	18	6[b]	2[b]	
Chi-Square		5.416	8.744	13.35	-
P value		0.144	0.032	0.004	-

Values within a column followed by a common letter were not significantly different (Beasley, 1995; post-hoc test).

Table 9. Taxis response of *O. arenosella* female moths to different blends at 20 µl in wind tunnel.

Source	Replications	TF	UF	SA	SC
BHC 1	20	9	6	3	0
BHC 20	20	8	6	7	0
BHC 24	20	9	9	6	0
Control (Solvent)	20	9	5	2	
Chi-Square		0.982	4.522	6.013	-
P value		0.806	0.21	0.111	-

Values within a column followed by a common letter were not significantly different (Beasley, 1995; post-hoc test).

The behaviour of herbivorous insects is often integrated with their host plants in a range of ways. This integration can be apparent from the effects induced by host plants on insect physiology and behaviour. Host plant volatiles synergize responses of sex pheromone-specific olfactory receptor neurons in male *Helicoverpa zea* and increase the responses (Ochieng *et al.,* 2002). Particularly important are the effects of host plants on pheromone behaviour, which appear to be a part of male strategies (to maximize encounters with females) as well as female strategies to gain access to new feeding and oviposition sites. The enhancement of sex attraction induced by host odours suggests and more effective traps can be devised for the management of insect pests (Reddy and Guerro, 2005). Present study results clearly indicated that addition of plant volatiles to sex pheromone performed better formulation than sex pheromone alone. Similar studies corroborative with present results, lures based solely on synthetic pheromones are unlikely to be fully competitive with signals emanating from food or plants (Reddy and Guerro, 2004). Based on this strategy, lures developed phenethyl euginol with sex pheromone for the Japanese beetle *Popillia japonica* Newman (Klein *et al.*, 1981), the dried-fruit beetle *Carpophilus lugubris* Murray aggregation pheromone plus host food odour (Lin *et al.*, 1992), the palm weevil *Rhynchophorus phoenicis* L. for ethyl propionate and pheromone (Gries *et al.,* 1994) and mixtures

of GLVs from cabbage (*Brassica oleracea*) and the pheromone a mixture of (Z)-11-hexadecenal, (Z)-11-hexadecenyl acetate, (Z)-11-hexadecenol, induced a significantly higher attractant and arresting behaviour in unmated males of the diamondback moth *Plutella xylostella* L. than the pheromone alone (Reddy *et al.,* 2002). Field baits of (Z)-3-hexenyl acetate and the pheromone enhanced the number of females caught in traps several folds over those baited with the natural attractant alone (Reddy and Guerrero, 2000). At a low release rate (1: 1000, sex pheromone: host plant volatile) of pheromone, addition of (E)-β-caryophyllene, (Z)-3-hexenyl acetate, 1-hexanol, or 1-octen-3-ol increased all behavioural responses, from activation to pheromone source contact (Arx *et al*., 2012).

Overall, the electrophysiological and behavioural studies designated that host odours linalool and acetophenone guide to *O. arenosella* to find out their host for oviposition and mate finding. Mixing with female sex pheromone can better perform (BHC 20 and BHC 24) in new blends compared to standard sex pheromone blend (BHC 1). Therefore, BHC 20 and BHC 24 can be recommended for *O. areosella* management. However, before the strong conclusion further studies can be suggested such as field mass trapping studies.

Acknowledgement

Authors would like to acknowledge the staff, Entomology Division, CPCRI, Kasargod, the staff, BCRL (PCI) pvt Ltd., Bangalore and the staff, Department of Entomology, University of Agricultural sciences, GKVK, Bangalore, India, who helped during the study.

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