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Volatile organic compounds in healthy and *Opisina arenosella* Walker (Lepidoptera: Oecophoridae) infested leaves of coconut palms

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ABSTRACT: An exploratory study, for the first time, was conducted to identify the volatile organic compounds (VOCs) present in dichloromethane extracts of uninfested and *O. arenosella* infested leaves of three varieties of coconut palms, viz. WCT, MGD and COD, as well as from the frass produced by the caterpillars while feeding on these varieties. Many VOCs reported from other plant species and having specific functions in plant-plant and plant-insect interactions were identified in the leaf material. Green leaf volatiles (Z)-3-hexen-1-ol and (Z)-3-hexen-1-ol acetate, acetophenone and nonanal were found in both uninfested and infested leaves. Differences in VOCs were observed between different varieties of coconut plants and between infested and uninfested plants of these varieties. The VOCs identified in insect frass included 6-hydroxy-2-hexanone, n-hexadecanoic acid, β-pinene, β-myrcene, hexahydrofarnesyl acetone, acetophenone and undecane. Indicated possible roles of the identified VOCs based on existing reports for other plant species.

KEYWORDS: GC-MS analysis, green leaf volatiles, herbivore-induced plant volatiles

INTRODUCTION

The coconut palm, *Cocos nucifera* L., is regarded in tropical countries as the 'Tree of Life' (Foale, 2003). The coconut cultivation industry in India directly or indirectly employs approximately 12 million people and contributes US $1.28 billion to the GDP of the country (Thomas, 2013). Coconut production is threatened by more than 800 species of pests in India and Sri Lanka (Singh and Rethinam, 2006). *Opisina arenosella* Walker (Lepidoptera: Oecophoridae), the black-headed caterpillar pest of coconut, is a serious defoliator of coconut palms in the entire coastal belt of Kerala and many parts of the states of Tamil Nadu and Karnataka. In severe outbreaks, thousands of palms can be affected with as much as 90% of leaf damage (Cock and Perera, 1987; Mohan and Sathiamma, 2007) and crop losses up to 45.4% in terms of nut yield from infested palms in the succeeding year of

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severe pest incidence (Chandrika *et al.*, 2010). Current control methods include chemical control by using pesticides such as monocrotophos or dicrotophos (Rao *et al.*, 1981) and biological control by releasing larval (*Goniozus nephantidis* Muesebeck), prepupal (*Elasmus nephantidis* Rohwer) and pupal parasitoids (*Brachymeria nosatoi* Habu) (Sathiamma *et al.*, 1987). However, an alternative and environmentally friendly approach is to exploit the plants' own defence mechanisms against herbivores.

The leaves of all plants normally release small quantities of volatile organic compounds (VOCs) known as constitutive volatiles. However, upon herbivore attack, the quantity and composition of volatile blends will change leading to the emission of herbivore-induced plant volatiles (HIPVs) (Pare and Tumlinson, 1999; Arimura *et al.,* 2009). Common VOCs emitted by plants include the 'green-leaf volatiles' (C6 aldehydes, alcohols, and derivatives), cyclic and acyclic terpenes, phenolics (aromatics), and sulphur- or nitrogen-containing compounds (Dicke, 1999; Dudareva *et al.,* 2004; Arimura *et al.,* 2009). These volatiles play multiple roles in plant defense, including herbivore repellence and deterrence, attraction of natural enemies, allelopathy and protection against abiotic stressors such as radiation and harmful gases (Holopainen, 2004; Kant *et al.*, 2009; Hare, 2011).

There is a great variability in the composition of volatile blends between plant species, and even among different plant genotypes and varieties within the same plant species (Gouinguene *et al.*, 2001; Degen *et al.*, 2004). Volatile emission is dynamic, changing through the course of the herbivory, and can vary depending on the herbivore species attacking, its abundance and developmental stage (De Moraes *et al.*, 1998; Girling *et al.*, 2011; McCormick *et al.*, 2014). Both herbivores and their natural enemies make use of the information conveyed by volatile blends to locate and infer the quality of their host plant or prey (Bruce *et al.*, 2005; Dicke, 2000; McCormick *et al.*, 2012; Mumm and Dicke, 2010).

The use of VOCs, especially the HIPVs, has gained importance in modern agricultural pest management practices (Yu *et al.*, 2008; Orre *et al.*, 2010). For example, in sweet corn, HIPVs attracted many parasitoids belonging to Eulophidae and Encyrtidae, which significantly reduced the sweet corn pest *Helicoverpa* spp. infestation (Simpson *et al.*, 2011). Information on the VOCs released by healthy and infested coconut palms and coconut pests is scarce and only few studies have been done (Bakthavatsalam *et al.*, 1999; Shameer *et al.*, 2002; Subaharan, 2008). Hence, an exploratory study was conducted to identify the volatile organic compounds (VOCs) present in healthy/ uninfested and *O. arenosella* infested leaves of three varieties of coconut palms, viz. WCT, MGD and COD, and the frass of larval galleries on the leaves of these varieties of coconut palms. Many VOCs have been identified and reported for the first time on these varieties of coconut palms. We reviewed the literature to discuss possible roles of these VOCs.

MATERIALS AND METHODS

Coconut palms

Three varieties of coconut palms, viz. West Coast Tall (WCT), Malayan Green Dwarf (MGD) and Chowghat Orange Dwarf (COD) were used for the present study. Two to three-years-old healthy coconut seedlings of WCT, MGD and COD varieties bearing 3-4 leaves were procured from the State Agriculture Department Coconut Nursery at Anakkayam, Kerala. The seedlings were transferred into clay pots (35 x 35 cm) with rich nutrient soil. Dried and powdered cow dung (bio-fertilizer) (250 gm) and a fertilizer mixture (100 gm of N, P and K in the proportion of 40-40-20) (Manufactured by MCP Agro Technologies (P) Ltd., Kerala and procured from the Fertilizer Depot of FACT, Kerala) were added to each seedling at 6-month intervals. The seedlings were watered daily and kept in an insect-proof net house.

Rearing *O. arenosella* in the laboratory

The larvae of all instars of *O. arenosella* collected from infested palms in the field were transferred into 500 ml glass beakers (Borosil, India) with freshly cut coconut leaves and covered with cotton cloth fastened with rubber bands. The larvae were transferred into new beakers every forty-eight hours with freshly cut coconut leaves till pupation and the pupae were kept in 250 ml glass beakers (Borosil, India) until the adults emerged. The emerged moths were allowed to mate for one day and released into caged leaves of coconut seedlings to maintain the insect rearing.

Since *O. arenosella* moths prefer to lay eggs in the larval galleries made of silken threads and frass, infested leaves with galleries collected from the field were cut into pieces (10-15 cm long) and 6-8 pieces of old galleries were pinned on the under surface of the undetached coconut leaf on the seedling. The leaf was enclosed within the net cage with the open end of the cage tied to the petiole of the leaf with jute twine and mated *O. arenosella* moths were introduced into the net cage. After 30 days of releasing the moths, the leaf was cut at the base of the petiole and larvae were carefully transferred into 500 ml glass beakers with fresh pieces of leaves. The larvae were transferred every two days to sterilized beakers with fresh pieces of leaves and seventh instar larvae were used for the experiments.

Extraction of volatile organic compounds from leaves of coconut palms

Collection of volatiles from healthy leaves

Fresh leaflets from 3-4 years-old coconut seedlings were cut at the base, washed in running water and cleaned with absorbent cotton to remove dust particles, if any. The leaflets were soaked in HPLC-Grade Dichloromethane (99.5%, Merck, India) for 12 hours (10 g in 50 ml DCM) (Scascighini *et al.*, 2005). The solvent extract was concentrated to 2 ml in Rotor Evaporator and filtered and decolorized by passing through Glasswool – anhydrous Sodium Sulphate (Na_2SO_4) - activated Charcoal column. The clear extracts were taken in Agilent glass tubes (2 ml) with Teflon caps (Agilent Technologies, U.S.A.) and stored at –60° C till analysis of the samples. The extraction was done simultaneously from the leaves of five individual plants of each of the three varieties of coconut.

Collection of volatiles from *O. arenosella* infested leaves

Twenty-five seventh instar *O. arenosella* larvae were introduced into a caged fresh leaf. The larvae were allowed to feed for 24 hours. After removing the frass and larvae carefully with a paintbrush, the volatiles were extracted from the infested leaves of three varieties of coconut palms separately using the same procedure as followed in the case of healthy leaves (Scascighini *et al.*, 2005).

Collection of volatiles from Larval Frass

The larval frass removed from the fed leaves of three varieties of coconut palms was separately soaked in DCM in the same proportion as done in the case of uninfested and infested leaves.

The extracts collected were labeled as follows -

WUL – WCT Uninfested Leaf; WIL – WCT Infested Leaf; WLF – WCT Larval Frass (Larval frass of *O. arenosella* on WCT); MUL – MGD Uninfested Leaf; MIL – MGD Infested Leaf; MLF – MGD Larval Frass (Larval frass of *O. arenosella* on MGD); CUL – COD Uninfested Leaf; CIL – COD Infested Leaf; CLF – COD Larval Frass (Larval frass of *O. arenosella* on COD).

Identification of volatiles in Gas Chromatography coupled Mass Spectrometry (GC-MS)

Five replicates of each variety of uninfested and infested leaves and frass of larvae were run in the GC-MS, and we only report compounds present in all replicates. One µl of the concentrated extract was injected in splitless mode into Agilent Gas Chromatograph (Model GC6890 N coupled with a HP 5975 B Mass Selective Detector). HP 5 Column was used with helium as a carrier gas.

During the run the temperature programme was as follows: 40°C for 3 min and rise at 5°C / min to 280° C, isotherm for 10 min; Post run 10 min at 300° C. The injector and column temperatures were 250° C. The total run was for 23 min. The retention time and mass spectra were compared with MP and NIST libraries. For compound identification, we report the best match with the NIST library, however, compound identity needs to be further confirmed against authentic standards.

RESULTS

A number of compounds belonging to various groups like green leaf volatiles, hydrocarbons, alcohols, aldehydes, acids, esters, ketones, terpenes, aromatic compounds etc. were identified in each variety of coconut palms. All the compounds appeared at retention times between 4.212 min. and 19.21 min.

Uninfested leaves of WCT, MGD and COD varieties

The uninfested leaves of all the three varieties of coconut palms produced a C6 compound, which is also a green leaf volatile, viz. (Z)-3-Hexen-1-ol. Whereas, other green leaf volatiles viz. (Z)-3-Hexen-1-ol acetate was present in MUL and CUL, and 2,4-dimethyl-Hexane was present in MUL. Heptacosane, a long chain hydrocarbon appeared in the compounds present in WUL and CUL. Apart from these, 2-Hexen-4-olide, Phenyl ethyl Alcohol, Isopropyl Myristate and Hexahydrofarnesyl acetone were obtained from WUL. β-Myrcene, a natural organic hydrocarbon classified as monoterpene, was present in MUL. Nonanal (a C9 aldehyde), Acetophenone and 1-Docosene were present in CUL (Table 1).

Table 1. Volatile organic compounds obtained in GCMS analysis of uninfested leaves of different varieties of coconut

Variety of coconut	RT (min.)	Compound	Class of compound
WUL	5.279	(Z)-3-Hexen-1-ol	Green leaf volatile
	6.324	2-Hexen-4-olide	Other
	7.92	Phenylethyl Alcohol	Alcohol
	13.128	Isopropyl Myristate	Ester
	13.309	Hexahydrofarnesyl acetone	Ketone
	16.249	Heptacosane	Long chain hydrocarbon
MUL	5.279	(Z)-3-Hexen-1-ol	Green leaf volatile
	6.696	β-Myrcene	Monoterpene
	6.792	(Z)-3-Hexen-1-ol acetate	Green leaf volatile
	7.056	o-Cymene	Aromatic
	7.678	2,4-dimethyl- Hexane	Other
CUL	5.274	(Z)-3-Hexen-1-ol	Green leaf volatile
	6.791	(Z)-3-Hexen-1-ol acetate	Green leaf volatile
	7.497	Acetophenone	Aromatic
	7.755	Nonanal	Aledhyde
	16.267	Heptacosane	Long chain hydrocarbon
	17.938	1-Docosene	Long chain hydrocarbon

RT - Retention Time, WUL – WCT Uninfested Leaf, MUL – MGD uninfested Leaf, CUL – COD Uninfested Leaf

Opisina arenosella infested leaves of WCT, MGD and COD varieties

(Z)-3-Hexen-1-ol and Heptacosane were obtained from the infested leaves of WIL and MIL, whereas Methyl Benzoate was present in WIL. (Z)-3-Hexen-1-ol acetate, Heneicosane, Nonanal and Decanal were isolated from MIL. 2,6-Dimethoxy benzoquinone was obtained from both MIL and CIL and aromatic compounds like p-Cymene and Acetophenone were obtained from CIL (Table 2).

Larval frass of *O. arenosella* reared on leaves of WCT, MGD and COD varieties

Monoterpenes like β-Pinene were found in WLF and CLF, whereas β-Myrcene was obtained from MLF. Aromatic compounds like p-Cymene was obtained from WLF and CLF and m-Cymene, o-Cymene and Acetophenone, were present in MLF and CLF. Compounds which were earlier reported as pheromone components of various other insects were also obtained from the frass of *O. arenosella*

Table 2. Volatile organic compounds obtained in GCMS analysis of *O. arenosella* infested leaves of different varieties of coconut

Variety of coconut	RT (min.)	Compound	Class of compound
WIL	4.657	Cyclobutene, 2-propenylidene	Other
	5.269	(Z)-3-Hexen-1-ol	Green leaf volatile
	7.745	Methyl benzoate	Aromatic
	7.855	Cyclohexane, 2-ethenyl-1, 1-dimethyl-3-methylene	Other
	15.527	Pterin-6-carboxylic acid	Carboxyilic acid
	16.25	Heptacosane	Long chain hydrocarbon
MIL	5.269	(Z)-3-Hexen-1-ol	Green leaf volatile
	6.789	(Z)-3-Hexen-1-ol, acetate	Green leaf volatile
	7.752	Nonanal	Aledhyde
	8.667	Decanal	Long chain aldehyde
	11.609	2,6-Dimethoxy benzoquinone	Other
	13.3	Hexahydrofarnesyl acetone	Ketone
	13.76	Methyl palmitate	Ester
	18.055	Heneicosane	Long chain hydrocarbon
	19.21	Heptacosane	Long chain hydrocarbon
CIL	6.786	p-Cymene	Aromatic
	7.489	Acetophenone	Aromatic
	7.749	1-Heptanol, 2-propyl-	Other
	11.765	2,6-Dimethoxy benzoquinone	Other
	14.035	n-Hexadecanoic acid (palmitic acid)	Fatty acid
	15.266	Oleic acid	Fatty acid

RT - Retention Time, WIL – WCT Infested Leaf, MIL – MGD Infested Leaf, CIL – COD Infested Leaf

Table 3. Volatile organic compounds obtained in GCMS analysis of larval frass of *O. arenosella* reared on different varieties of coconut

Variety of coconut	RT (min.)	Compound	Class of compound
WLF	4.212	3-methyl-3-Hexanol	Other
	4.368	Cyclobutene, 2-propenylidene	Other
	6.664	β-Pinene	Monoterpene
	7.049	p-Cymene	Aromatic
	13.306	Hexa -hydrofarnesyl acetone	Ketone
	13.554	Hexa-hydro-farnesol	Alcohol
MLF	5.645	6-hydroxy-2-Hexanone	Other
	6.707	β-Myrcene	Monoterpene
	7.052	m-Cymene	Aromatic
	7.49	Acetophenone	Aromatic
	13.309	Hexahydrofarnesyl acetone	Ketone
CLF	6.673	β-Pinene	Monoterpene
	6.751	p-Cymene	Aromatic
	7.05	o-Cymene	Aromatic
	7.49	Acetophenone	Aromatic
	7.675	Undecane	Long chain hydrocarbon
	8.314	Methyl nicotinate	N containing compound
	14.079	n-Hexadecanoic acid (palmitic acid)	Fatty acid
	15.267	Oleic acid	Fatty acid
	15.419	Phytol	Diterpene alcohol

RT - Retention Time, WLF – Larval Frass on WCT, MLF – Larval Frass on MGD, CLF – Larval Frass on COD

reared on different varieties of coconut plams. Hexa -hydrofarnesyl acetone and Hexa-hydro-farnesol, were obtained from WLF; 6-hydroxy-2-Hexanone, was obtained from MLF and the long chain hydrocarbon, Undecane, was obtained from CLF (Table 3).

DISCUSSION

The present study revealed the presence of many potential VOCs in healthy and *O. arenosella* infested leaves of three varieties of coconut palms and frass of the larvae of *O. arenosella.* Though compound identification was based on the best match to the NIST library, compounds could be accurately classified to chemical group level (e.g. green leaf volatile or terpenoid) by the characteristics of their mass spectra. Since many VOCs in WCT, MGD and COD varieties of coconut palms are reported for the first time in the present study, e.g. β-Myrcene in MUL and Nonanol in CUL, previous references are lacking to check whether these compounds are released by all the varieties.

Some compounds, like (*Z*)-3-hexenol are present in uninfested leaves of all varieties, whereas others such as (*Z*)-3-hexenyl acetate are found in two of the three varieties (MUL and CUL). Other compounds appear to be unique to a specific variety, for instance β-myrcene in MUL or nonanol in CUL, indicating that there are varietal differences in the odour profiles (Table 1). The herbivore-induced profiles show even more differences among varieties (Table 2). Similar differences on healthy and infested leaf volatile profiles have been reported for other plant species like cotton (Loughrin *et al.,* 1995) and barley (Pettersson *et al.,* 1999). The volatiles emitted from the vegetative parts of plants, especially those released after herbivory, may protect plants by deterring herbivores and by attracting the natural enemies of herbivores (Pichersky and Gershenzon, 2002).

(Z)-3-Hexen-1-ol, (Z)-3-Hexen-1-ol acetate and 2,4-dimethyl- Hexane, which were obtained from the leaves of coconut palms are Green Leaf Volatiles (GLVs), C_6 aldehydes, esters and carbons released after mechanical wounding (Arimura *et al.,* 2009). These GLVs, produced or emitted upon herbivory by almost every green plant, are used by insects as semiochemicals and are reported to be involved in indirect and direct plant defenses of many plant species (Scala *et al*., 2013). Subaharan (2008) reported that (Z)-3-hexen-1-ol was present in both damaged and undamaged leaves of coconut palms. Matsui *et al.* (2012) have reported that (Z)-3-hexenol and (Z)-3-hexenyl acetate are produced in the intact parts of partially wounded leaves. The green-leaf volatile, (Z)-3-hexenyl acetate, and other aliphatic esters of (Z)-3-hexen-1-ol that are emitted by tobacco after damage were found to deter female *Heliothis virescens* moths from laying eggs on injured plants (Moraes *et al*., 2001). Several examples of (Z)-3-hexenyl acetate-mediated plant communication have also been reported (Prost *et al.*, 2005; Engelberth *et al.*, 2004; Heil and Kost, 2006).

Terpenes are also an important group of plant volatiles serving multiple roles in plant defense such as antimicrobial activities, protection against abiotic stress and plant-insect communication (Cheng *et al.*, 2007; Gershenzon and Dudareva, 2007; Pichersky *et al*., 2006). The uninfested leaves of MGD variety produced β-Myrcene, which is a monoterpene and a common constitutive volatile of many plants having possible antimicrobial activity (Laouer *et al.,* 2009). The frass of *O. arenosella* larvae also contained monoterpenes like β-Pinene and β-Myrcene. Both are common constitutive volatiles produced by plants and possibly involved in antimicrobial activity, which possibly remain in the larval frass after the ingestion of plant material (Dorman and Dean, 2000; Laouer *et al.,* 2009). Subaharan (2008) reported the presence of α-pinene in damaged leaves and larval frass, and β-pinene in undamaged and damaged leaves, but the variety of coconut palm used for the study is unknown. Terpenoids can also play a role in indirect defense by attracting natural enemies of herbivores, for example feeding by *Pieris rapae* larvae induces *Arabidopsis thaliana* to emit a blend of volatiles consisting of terpenoids that is attractive to a larval parasitoid of *P. rapae* (Van Poecke *et al*., 2001).

Aromatic compounds have simple aromatic rings and C1–C3 side, one of the most important representatives of the group is methyl salicylate which plays important roles in plant defense and signaling (Loake and Grant, 2007; Park *et al*., 2007). Members of this group such as Acetophenone, Methyl Benzoate and p-Cymene appeared in the infested leaf samples. Acetophenone is reported to be a possible insect attractant in the flowers of red clover plant *(Trifolium pratense* L.) (Buttery *et al.,* 1984), whereas Methyl Benzoate is a common floral volatile involved in the pollinator attraction, e.g. in common garden snapdragon, *Antirrhinum majus* (Pischerski and Gershenzon, 2002) and p-Cymene was reported to have a possible role in plant-insect interactions, e.g. it acts as a repellent in tomato (*Solanum lycopersicum*) against the whitefly, *Bemisia tabaci* (Bleeker *et al.,* 2009). Aromatic compounds like Acetophenone, p-Cymene, m-Cymene and o-Cymene were also obtained from the frass of *O. arenosella*. m-Cymene and o-Cymene have been reported as having antioxidant and antimicrobial properties in *Citrus acida* Roxb. against bacteria such as *Bacillus subtilis* and fungi such as *Candida utilis* (Mahmud *et al.,* 2009).

Insect-derived hydrocarbons have major ecological roles mediating species- and gender- recognition and nest mate recognition; and are used as task-specific cues, dominance and fertility cues, and primer pheromones (Howard and Blomquist, 2005).

For instance, Heptacosane has been reported to be a sex pheromone component of several insect species (El-Sayed, 2016), and Heneicosane, was also reported as a pheromone for attracting the female *Aedes aegypti* (Bhutia *et al*., 2010). Long chain hydrocarbons can also be emitted by plants and mediate plant-insect interactions. For instance, Heneicosane, obtained from healthy and infested leaves during the present study has been previously reported in maize volatile extracts which mediate plant-insect interactions (Krokos *et al*., 2002), whereas another long chain hydrocarbon, 1-Docosene, identified in healthy leaves is reported to be a floral volatile and possible insect attractant in *Clusia* (Guttiferae) plants (Nogueira *et al*., 2001).

Long chain aldehydes like nonanal appeared in both un-infested and infested leaves and decanal appeared in the infested leaf only. Nonanal has been frequently reported as an important component of volatile blends involved in insect attraction (Cha *et al*., 2008; Fraser *et al*., 2003; Metcalf and Kogan, 1987). Other examples are: nonanal, released from whitefly infected beans (*Phaseolus vulgaris*) (Birkett *et al*., 2003), was also reported to induce resistance against bacterial pathogen, *Pseudomonas syringae* in Lima bean (*Phaseolus lunatus*) plants (Yi *et al.,* 2009). Nonanal and Decanal released from apple trees elicited high EAG response in *Cydia pomonella* (Gonzalez, 2007).

Compounds belonging to other chemical classes were also found in the frass and leaf material. For instance, the infested leaves of MGD and COD varieties produced 2,6-dimethoxy benzoquinone, which is reported to be involved in plant-plant communication, e.g. it induces haustorium development in parasitic plants in the *Scrophulariaceae* to invade the roots of neighboring plants (Yoder, 2001) and can also have antibiotic activities ((Nishina *et al*., 1991), e.g. 2,6-dimethoxy-1,4-benzoquinone enhances resistance against rice blast fungus *Magnaporthe oryzae* (Ueno and Yoshikiyo, 2014). Frass samples contained Hexa-hydro-farnesyl acetone and Hexa-hydro-farnesol which have been reported as pheromone components reported from many Hymenopteran insect species, such as *Geotrigona mombuca*, *Apis dorsata* (both Apidae) (El-Sayed, 2016). Hexa-hydro-farnesyl acetone was also reported as an attractant in male orchid bee, *Euglossa* spp. (Eltz *et al.,* 2010) and also reported to induce antimicrobial activity in the Algerian *Phlomis bovei* De Noé against pathogens (Liolios *et al*., 2007).

It is evident from the present study that the leaves of coconut palms produced many VOCs, which have been reported to have important functions in other plants and insects. Many of the VOCs produced by uninfested and infested coconut palms and frass are herbivore-induced plant volatiles (HIPVs), that can mediate both direct and indirect defenses acting as herbivore deterrents, or attracting the foraging carnivorous predators and parasitoids to kill the herbivores (De Moraes *et al.,* 2001; Dicke *et al*., 2009; Mumm and Dicke, 2010; McCormick *et al*., 2012).

Ghosh and Abdurahiman, as early as 1996, suggested that the kairomones emitted from the larval gallery of *O. arenosella* might serve as an important factor in host searching and oviposition behaviour of early larval parasitoid *Apanteles taragamae* Viereck (Hymenoptera: Braconidae). Subaharan (2008) reported that the damaged coconut leaflet odors elicited more antennal response in the late larval parasitoid *Goniozus nephantidis* Muesebeck (Hymenoptera: Bethylidae) than the undamaged leaflets, however the identity of the compounds and blends responsible for this response remains unknown.

The role of VOCs produced by coconut palms in either direct or indirect defence has not been investigated so far and hence, we cannot attribute any functions to particular VOCs identified from the coconut leaves. However, earlier reports on other plants and insects suggest that the VOCs identified from coconut leaves might have important roles in coconut palm – *O. arenosella* – interactions with herbivores and their natural enemies. Further studies are required to investigate the differences between the volatiles profiles of infested and uninfested plants, and behavioral assays with herbivores and their natural enemies are required to establish the role of the identified compounds in the interactions between the coconut palms and the insect community.

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