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Acaricidal and repellent activities of *Litsea cubeba* (Lour.) oil and 3,7-dimethyl-2,6-octadienal against *Haemaphysalis longicornis* (Acari: Ixodidae)

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Abstract

The bioactivity of the essential oil extracted from *Litsea cubeba* fruits against tick vectors of severe fever with thrombocytopenia syndrome is unknown. In this study, *L. cubeba* oil and its main constituents, 3,7-dimethyl-2,6-octadienal and its similar structures, were evaluated for their acaricidal and repellent activities on the unfed nymphs and adults of *Haemaphysalis longicornis*. *L. cubeba* oil displayed both acaricidal and repellent activities against both life stages. Among the constituents of *L. cubeba* oil, only 3,7-dimethyl-2,6-octadienal exhibited both acaricidal and repellent activities against both life stages. In a repellent bioassay, 3,7-dimethyl-2,6-octadienal and *L. cubeba* oil at dose of 0.08 mg/cm^2 provided excellent repellence (100%) against the nymphs and adults for over 60 min post-application. When the acaricidal and repellent activities of 3,7-dimethyl-2,6-octadienal and its similar structures were compared, activities of all tested derivatives were significantly less potent than those of 3,7-dimethyl-2,6-octadienal. The strong acaricidal and repellent activities of 3,7-dimethyl-2,6-octadienal in *L. cubeba* oil suggests that it is a promising natural candidate for developing new sustainable acaricidal and repellent agents.

Keywords: Acaricidal activity, *Haemaphysalis longicornis*, *Litsea cubeba* fruits, Repellent activity

Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease that is clinically characterized by thrombocytopenia, gastrointestinal symptoms, and high fever [1]. SFTS is caused by the SFTS virus, a member of the genus *Phlebovirus* (family *Bunyaviridae*), which was first discovered in China in 2009 and then subsequently found in Japan and South Korea in 2013 [1–3].

The hard tick *Haemaphysalis longicornis* (Acari: Ixodidae), which is widely distributed in Japan, China, Korea, and Oceania [1, 2], is regarded as the primary vector of the SFTS virus [1, 2]. *H. longicornis* is the most abundant tick species in South Korea, attacking both livestock and humans, with infestation rates peaking between May and September [3]. Although the incidence of SFTS is low in comparison to other tick-borne diseases, it remains a serious public health issue, causing widespread public concern in South Korea due to its high mortality rate. Unfortunately, no vaccine or medication for SFTS is currently available, and with the population of *H. longicornis* rapidly increasing due to global warming, SFTS virus-infected *H. longicornis* may pose a global threat in the near future. Thus, it is necessary to protect people and

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livestock from infected ticks. At present, the control of *H. longicornis* depends heavily on the use of synthetic acaricides. However, the indiscriminate and frequent overuse of synthetic acaricides can lead to environmental contamination, non-target toxicity, and widespread development of resistance [4]. As a result, many researchers are working to develop environmentally friendly acaricides to protect people and livestock from ticks, and they have discovered that many plant-derived substances may be used as new acaricides for tick control [4–6].

The current study focuses on *Litsea cubeba* (Lour.) Pers. (Lauraceae), which is widely distributed in North America, Japan, Southern China, and Southeast Asia [7]. Various extracts from different parts of *L. cubeba* have been used to treat ailments in traditional Chinese folk medicine [8]. It has also been demonstrated that *L. cubeba* oil has insecticidal and repellent activity against insects such as the cigarette beetle (*Lasioderma serricorne)*, booklouse (*Liposcelis bostrychophila)*, and mosquito *Aedes aegypti*) [7, 9].

To the best of our knowledge, no studies on the acaricidal and repellent properties of *L. cubeba* oil against *H. longicornis* have been conducted. Therefore, this study was carried out to investigate the chemical composition of *L. cubeba* oil and to evaluate its efficacy in the control of *H. longicornis* in terms of its acaricidal and repellent properties. Our findings may offer new insights into the potential future use of *L. cubeba* oil and its constituents to design effective and environmentally friendly acaricidal and repellent formulations.

Materials and methods

Chemicals

1,8-Cineole (99%), 3-[*N*-butyl-*N*-acetyl] aminopropionic acid ethyl ester (IR3535) (95%), 3,7-dimethyl-2,6-octadienal (96%), (±)-limonene (97%), 3,7-dimethyl-6-octenal (95%), 2-octenal (95%), octanal (99%), 2,4-octadienal (95%), α-pinene (98%), and β-pinene (99%) were purchased from Sigma-Aldrich (Missouri, MO, USA).

Sample preparation

Fruits of *L. cubeba* grown in China were obtained from the herb market in Jeonju, Republic of Korea. Essential oil was obtained by steam distillation for 8 h in a Clevenger-type apparatus, as previously described [10]. The essential oil was dehydrated with anhydrous magnesium sulfate and evaporated to dryness using a rotary evaporator (EYELA, Tokyo, Japan) at 28 °C.

Test ticks

H. longicornis unfed nymphs and adults were collected from cattle farms in Seogwipo-si, Jejudo, Republic of Korea. The collected nymphs and adults were morphologically identified as *H. longicornis* according to Baker and Walker [11].

Essential oil analysis

The essential oil of the *L. cubeba* fruits was analyzed using a GC–MS (HP 6890 GC and 5973 Mass Selective Detector, Agilent Technologies, Santa Clara, CA, USA) equipped with a fused-silica capillary column (DB-5: 30 m × 0.25 mm inner diameter (i.d.) × 0.25 μm thickness, Agilent Technologies). The initial oven temperature (60 °C) was raised to 210 °C at a rate of 2 °C/min and held there for 10 min. The oven temperature was then raised to 240 °C at a rate of 2 °C/min for 15 min. Helium was used as the carrier gas at 0.8 mL/min. Spectra were obtained at an ionization energy of 70 eV, and the mass analyzer was set to scan from 50 to 550 amu. The constituents of *L. cubeba* oil were identified based on their retention index and retention time, and the mass spectra were compared with a mass spectrum library [12].

Acaricidal toxicity bioassays

The nymphal immersion test was performed on unfed *H. longicornis* nymphs, using the methodology described by Osman et al. [13]. Twenty unfed nymphs were immersed for 5 min in test solutions (10 mL) of the essential oil or individual compounds at concentrations of 2.5, 5, 10, 20, 40, 60, and 80 mg/mL. The negative control groups were immersed in 50% ethanol and 2% dimethyl sulfoxide (DMSO) (solvent). Following immersion, the unfed nymphs were placed in a Petri dish (1.5 cm deep × 5.5 cm i.d.) with filter paper (5.5 cm i.d. × 25 μm thickness, catalogue no. 1004–055, Whatman, Maidstone, UK) for 10 min to allow the solvent to evaporate. Petri dishes containing treated nymphs were incubated at 26–28 °C and 85%–90% relative humidity (RH) for 24 h.

Bioassays of unfed *H. longicornis* adults were carried out using the adult immersion test as previously described [13]. In each test solution of 2.5, 5, 10, 20, 40, 60, and 80 mg/mL, twenty unfed adults were immersed for 5 min, as in the nymphal immersion test. The negative control group was immersed in 50% ethanol and 2% DMSO. After immersion, the unfed adults were dried on Petri dishes with filter paper for 10 min, and the dishes were incubated at 27–28 °C at 80%–90% RH. All treatments were repeated three times. After 24 h, dead ticks were counted via microscopic examination. Mortality data of each treatment were corrected using Abbott's formula [14].

Repellency activity bioassays

To evaluate the effect of each test sample on the behavior of the unfed nymphs and adults, a filter paper bioassay described by Fabbro and Nazzi [15] was used with

slight modifications. In brief, two concentric circles with diameters of 3.0- and 7.0-cm (line A, start line and line B, finish line) were drawn with a pencil on filter paper (9.0 cm diameter, Whatman, Maidstone, UK) (Figure 1), and then placed on a Petri dish. One hundred microliters of ethanol (solvent) containing each test sample at a concentration of 0.08 mg/cm^2 or ethanol alone (negative control) was applied outside the finish line (line B) on the filter paper and dried for 10 min. IR3535 (a known repellent) was used as a positive control in repellency tests against *H. longicornis* unfed nymphs and adults. A single unfed nymph or adult was tested on the control filter paper first, and then on the test filter paper. Each tick was placed with a soft paint brush (5 mm wide) at the center of the arena. Ticks were observed until they crossed the finish line (line B), and the time taken to travel from the start line (line A) to the finish line (line B) was recorded. Ten bioassays against the test samples and 10 bioassays against the negative control (ethanol) were performed for each test. If the test sample was found to be repellent at 0.08 mg/cm^2, it was also tested at 0.04 mg/cm^2, and if it was repellent at 0.04 mg/cm^2, it was also tested at 0.02 mg/cm^2.

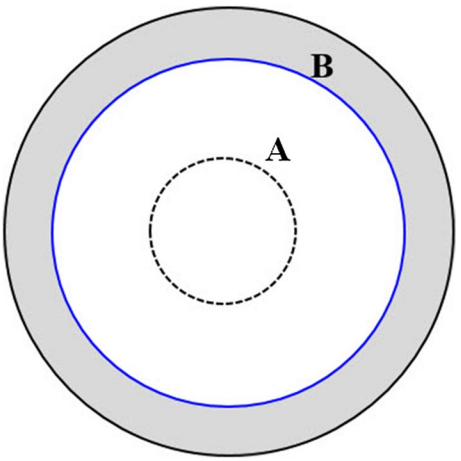

Figure 1 Filter paper arena used in the repellent bioassays. Two concentric circles (3.0 and 7.0 cm diameter, line **A**: start line and line **B**: finish line) were drawn by pencil on the filter paper. Each test sample or ethanol alone (negative control) was applied outside the finish line (dark grey) on the filter paper. A single unfed nymph or adult was first tested on the control filter paper, and subsequently on the filter paper with test samples. Each tick was placed with a soft paint brush (5 mm wide) in the center of the arena. Ticks were carefully observed until they crossed the finish line (line B), and the time required to travel from the start line (line A) to finish line (line B) was recorded

Statistical analysis

Mortality data from the acaricidal toxicity bioassays were subjected to Probit analysis for calculation of 50% and 90% lethal concentration (LC_{50} and LC_{90}) and their 95% confidential limits (CLs), and chi-square values using SPSS (version 12.0; SPSS, Chicago, USA). Results from the Petri dish bioassay were not normally distributed; therefore, the median was used as a measure of central tendency. To check for statistically significant differences between treatment and negative controls, the time spent to travel from lines A (start line) to B (finish line) was compared using the Mann–Whitney U test.

Results

Acaricidal toxicity and chemical composition of *L. cubeba* oil

Acaricidal toxicity of *L. cubeba* oil was evaluated in *H. longicornis* unfed nymphs and adults using the nymphal and adult immersion tests, respectively (Table 1). The LC_{50} values of *L. cubeba* oil against unfed nymphs and adults were 39.34 and 47.64 mg/mL, respectively. No tick mortality was observed in the negative control (50% ethanol + 2% DMSO). To further explore the acaricidal toxicities of *L. cubeba* oil against unfed nymphs and adults, the constituents of *L. cubeba* oil were analyzed by GC–MS, and the results are shown in Table 2. Thirteen constituents were identified in *L. cubeba* oil including 3,7-dimethyl-2,6-ctadienal (75.88%), (+)-limonene (10.44%), eucalyptol (3.70%), α-pinene (1.55%), and-β-pinene (1.02%). The other constituents (camphene, sabinene,

Table 1 Acaricidal activities of *Litsea cubeba* oil against *Haemaphysalis longicornis* unfed nymphs and adults

Samples	Developmental stage	LC_{50} (mg/mL) (95% CL)[a]	LC_{90} (mg/mL) (95% CL) [a]	Slope ± SE	χ^2 value (df, *p*)
Litsea cubeba	Nymphs	39.34 (34.25–44.31)	70.74 (61.76–92.81)	5.51 ± 0.89	4.714 (5, 0.452)
	Adults	47.64 (41.94–53.09)	78.99 (68.65–97.93)	5.84 ± 0.95	5.338 (6, 0.501)
Negative control	Nymphs	–[b]	–	–	–
	Adults	–	–	–	–

[a] LC_{50} (LC_{90}) is the average of three determinations, with 20 ticks per replication, exposed for 24 h

[b] No activity

Table 2 Identification of chemical components of *L. cubeba* oil

Compound	Retention Time (min)	Relative area (%)	Molecular mass (g/mol)	Molecular formula
α-Pinene	5.008	1.55	136.24	$C_{10}H_{16}$
Camphene	5.295	0.40	136.24	$C_{10}H_{16}$
Sabinene	5.720	0.72	136.24	$C_{10}H_{16}$
β-Pinene	5.810	1.02	136.24	$C_{10}H_{16}$
Methyl heptenone	5.880	0.93	126.20	$C_8H_{14}O$
(+)-Limonene	6.756	10.44	136.24	$C_{10}H_{16}$
Eucalyptol	6.913	3.70	154.25	$C_{10}H_{18}O$
α-Terpineol	9.679	0.69	154.25	$C_{10}H_{18}O$
3,7-Dimethyl-2,6-octadienal	10.505	75.88	152.24	$C_{10}H_{16}O$
Geraniol	10.652	0.76	154.25	$C_{10}H_{18}O$
Piperitone	10.758	0.45	152.24	$C_{10}H_{16}O$
4-Isopropylcyclohexanol	12.507	0.27	142.24	$C_9H_{18}O$
β-Caryophyllene oxide	15.594	0.40	204.36	$C_{15}H_{24}$
Total		97.21		

methyl heptenone, α-terpineol, geraniol, piperitone, 4-isopropylcyclohexanol, and β-caryophyllene oxide) accounted for less than 1%.

Acaricidal toxicity of the individual constituents

To determine the major constituent responsible for the toxicity, the acaricidal activities of the five major constituents (3,7-dimethyl-2,6-octadienal, eucalyptol, (+)-limonene, α-pinene, and β-pinene) were tested against the *H. longicornis* unfed nymphs and adults (Table 3). Among the five major constituents tested, only 3,7-dimethyl-2,6-octadienal exhibited acaricidal toxicities against unfed nymphs and adults, with LC_{50} values of 32.58 and 41.06 mg/mL, respectively. Eucalyptol, (+)-limonene, α-pinene, and β-pinene did not have acaricidal toxicity against unfed nymphs and adults, even at the highest concentration tested (> 80 mg/mL).

Repellent activity of *L. cubeba* oil and its individual constituents

The repellent activity of *L. cubeba* oil and its individual constituents against the *H. longicornis* unfed nymphs and adults was evaluated using a filter paper bioassay, and compared with that of the positive control (IR3535) (Table 4). *L. cubeba* oil demonstrated repellant activity against the unfed nymphs and adults at dosages of

Table 3 Acaricidal activities of the major constituents of *L. cubeba* oil against *H. longicornis* unfed nymphs and adults

Samples	Developmental stage	LC_{50} (mg/mL) (95% CL)[a]	LC_{90} (mg/mL) (95% CL)[a]	Slope ± SE	χ^2 value (df, *p*)
3,7-Dimethyl-2,6-octadienal	Nymphs	32.58 (27.75–37.84)	67.19 (55.41–88.11)	4.08 ± 0.56	6.162 (6, 0.405)
	Adults	41.06 (35.79–46.10)	71.27 (61.39–91.18)	5.35 ± 0.85	3.392 (6, 0.758)
Eucalyptol	Nymphs	> 80	–	–	–
	Adults	> 80	–	–	–
(+)-Limonene	Nymphs	> 80	–	–	–
	Adults	> 80	–	–	–
α-Pinene	Nymphs	> 80	–	–	–
	Adults	> 80	–	–	–
β-Pinene	Nymphs	> 80	–	–	–
	Adults	> 80	–	–	–
Negative control	Nymphs	–[b]	–	–	–
	Adults	–	–	–	–

[a] LC_{50} (LC_{90}) is the average of three determinations, with 20 ticks per replication, exposed for 24 h

[b] No activity

Table 4 Repellent activities of *L. cubeba* oil and its major components at different dosages against *H. longicornis* unfed nymphs and adults measured by filter paper bioassay

Samples	Developmental stage	Dose (mg/cm^2)	Treatment median (s)	Negative control median (s)	*P* value[a]	Treatment/ negative control[b]	Median difference (s)[c]	Relative repellency[d] IR3535
L. cubeba	Nymphs	0.08	120.5	14.5	< 0.01	8.3	106.0	3.3
		0.04	41.5	13.5	< 0.01	3.1	28.0	2.3
		0.02	17.5	13.0	n.s.[e]	1.3	4.5	1.8
	Adults	0.08	73.0	7.0	< 0.01	10.4	66.0	4.6
		0.04	32.0	7.5	< 0.01	4.3	24.5	3.5
		0.02	9.5	7.0	n.s	1.4	2.5	1.0
α-Pinene	Nymphs	0.08	22.0	14.0	n.s	1.6	8.0	0.3
	Adults	0.08	9.5	6.5	n.s	1.5	3.0	0.2
β-Pinene	Nymphs	0.08	25.0	15.5	n.s	1.5	9.5	0.3
	Adults	0.08	8.0	6.0	n.s	1.3	2.0	0.1
(+)-Limonene	Nymphs	0.08	18.0	14.0	n.s	1.1	4.0	0.1
	Adults	0.08	8.5	6.0	n.s	1.1	2.0	0.1
Eucalyptol	Nymphs	0.08	18.0	12.5	n.s	1.2	5.5	0.2
	Adults	0.08	10.0	7.5	n.s	1.1	2.5	0.2
3,7-Dimethyl-2,6-octadienal	Nymphs	0.08	160.5	16.0	< 0.01	10.0	144.5	4.5
		0.04	66.5	14.0	< 0.01	4.8	52.5	4.4
		0.02	20.0	13.5	n.s	1.5	6.5	2.6
	Adults	0.08	96.0	9.5	< 0.01	10.1	86.5	6.0
		0.04	51.5	6.0	< 0.01	8.6	45.5	6.5
		0.02	13.0	9.0	n.s	1.4	4.0	2.7
IR3535	Nymphs	0.08	45.5	13.5	< 0.01	3.4	32.0	1.0
		0.04	23.0	10.5	< 0.05	2.2	12.0	1.0
		0.02	12.5	10.0	n.s	1.3	2.5	1.0
	Adults	0.08	21.0	6.5	< 0.01	3.2	14.5	1.0
		0.04	14.0	7.0	< 0.05	2.0	7.0	1.0
		0.02	9.5	8.0	n.s	1.2	1.5	1.0

[a] Statistical significance of the difference between the treatment and negative control medians

[b] Treatment median (s)/negative control median (s)

[c] Median difference between treatment and negative control medians

[d] Median difference between treatment and negative control medians/median difference between positive control and negative control

[e] No statistically significant difference

0.04 and 0.08 mg/cm^2 ($P < 0.01$) when compared to the negative control. The median values of the time that individual ticks spent on *L. cubeba* oil treatments and the negative control at doses of 0.04 and 0.08 mg/cm^2 were 28.0 s and 106.0 s for unfed nymphs and 24.5 s and 66.0 s for unfed adults, respectively. Based on the median difference between treatment and negative control, the *L. cubeba* oil at doses of 0.04 and 0.08 mg/cm^2 was approximately 2.3 and 3.3 times more repellent to unfed nymphs and 3.5 and 4.6 times more repellent to unfed adults, respectively, when compared to IR3535. Among the five major constituents of *L. cubeba* oil tested against unfed nymphs and adults, significant differences ($p < 0.01$) between the time spent on treatment and the negative control were observed only for 3,7-dimethyl-2,6-octadienal at doses of 0.04 and 0.08 mg/cm^2 (Table 4). Eucalyptol, (+)-limonene, α-pinene, and β-pinene had no discernable effect on unfed nymphs and adults, even at the highest dose (0.08 mg/cm^2) tested. Based on the median difference between the treatment and negative control, the 3,7-dimethyl-2,6-octadienal at doses of 0.04 and 0.08 was approximately 4.4 and 4.5 times more repellent to unfed nymphs and 6.5 and 6.0 times more repellent to unfed adults, respectively, when compared to IR3535.

Acaricidal and repellent activities of 3,7-dimethyl-2,6-octadienal and its similar structures

According to our findings, the strongest acaricidal and repellent compound in *L. cubeba* oil is 3,7-dimethyl-2,6-octadienal. To investigate the structure–activity relationships of 3,7-dimethyl-2,6-octadienal, acaricidal and repellant activities of four compounds (3,7-dimethy-6-octenal, octanal, 2-octenal, and 2,4-octadienal) with chemical structures similar to 3,7-dimethyl-2,6-octadienal were tested. The acaricidal toxicities of the 3,7-dimethyl-2,6-octadienal and its similar structures were evaluated using nymphal and adult immersion tests (Table 5). Of the five compounds tested, only 3,7-dimethyl-2,6-octadienal with two carbon–carbon double bonds and methyl groups was shown to have acaricidal toxicity against the unfed nymphs and adults. 3,7-dimethyl-6-octenal (with a single carbon–carbon double bond and two methyl groups), octanal (with no carbon–carbon double bond and methyl group), 2-octenal (with a single carbon–carbon double bond), and 2,4-octadienal (with two carbon–carbon double bonds) did not exhibit acaricidal toxicity against the unfed nymphs and adults.

The repellent activities of 3,7-dimethyl-2,6-octadienal and its similar structures were determined at 0.08 mg/cm^2 using a filter paper bioassay (Table 5). Based on the median difference between the treatment and negative control, 3,7-dimethyl-2,6-octadienal (144.5 s and 86.5 s, respectively) exhibited the highest repellency against the unfed nymphs and adults, followed by 3,7-dimethyl-6-octenal (59.5 s and 34.5 s, respectively), 2,4-octadienal (15.0 s and 9.0 s, respectively), and 2-octenal (11.5 s and 6.5 s, respectively). Octanal had no discernable effect on the unfed nymphs and adults. The presence of carbon–carbon double bonds may play an important role in repellency, as 2,4-octadienal and 2-octenal with either one or two carbon–carbon double bonds were more repellent than octanal with no carbon–carbon double bond. Moreover, the strength of repellency increased as the number of carbon–carbon double bonds increased. The methyl groups also appeared to play a role as 3,7-dimethyl-2,6-octadienal and 3,7-dimethyl-6-octenal (with two methyl groups) were more repellent than 2,4-octadienal and 2-octenal with no methyl groups.

Discussion

The current study demonstrates that the essential oil of *L. cubeba* grown in China shows acaricidal and repellent properties against *H. longicornis* unfed nymphs and adults (Tables 1, 3). The main component composition of the *L. cubeba* oil observed here differed from that reported in previous studies [12]. The essential oil used in this study, which was extracted from *L. cubeba* fruit samples grown in China, was rich in 3,7-dimethyl-2,6-octadienal (75.88%) (Table 2), whereas samples cultivated in two different Indian locations were rich in citronellal (44.8–77.2%) and citronellol (10.9–14.0%) [16]. These changes in essential oil chemical composition can be attributed to a number of physical environmental factors (climate change and seasonal variation) as well as plant genetic differences [17]. Moreover, many studies have reported variations in the composition of major constituents of essential oils with respect to growth stage and geographical origin [18]. Consequently, the acaricidal and repellent effects of essential oils are affected by their growth stage and geographical origin, since the major constituents of the essential oils determine their unique biological activity [19].

The high composition levels of the major compounds may account for *L. cubeba* oil's superior acaricidal and repellent properties. Of the major constituents tested in *L. cubeba* oil, only 3,7-dimethyl-2,6-octadienal exhibited acaricidal and repellent activities against *H. longicornis* unfed nymphs and adults. As a result, 3,7-dimethyl-2,6-octadienal was identified as the active component of *L. cubeba* oil with significant acaricidal and repellent properties. Many researchers have reported that plant essential oils and their individual constituents show not only high acaricidal activity, but also potent repellent activity against mites and ticks [5, 20, 21]. There are few studies on the acaricidal and repellent activities of plant essential oils against *H. longicornis*. Nong et al. [6] and Yang et al. [7] conducted studies of plant essential oils reporting toxicity toward *H. longicornis* using oils extracted from aerial parts of *Eupatorium adenophorum* and the roots of *Morinda officinalis*, all of which had remarkable acaricidal effects, regardless of the bioassays used (fumigation, larval/nymphal immersion test). To the best of our knowledge, this is the first study to report the acaricidal and repellent properties of *L. cubeba* oil and its main constituent against *H. longicornis* unfed nymphs and adults.

Structure–activity relationships of acaricidal and repellent compounds against arthropod species have been well studied [15, 22, 23]. Interestingly, in 3,7-dimehtyl-2,6-octadienal and its similar structures tested in this study, 2-octenal, 2,4-octadienal, and 3,7-dimethyl-6-octenal demonstrated repellent activity against *H. longicornis* unfed nymphs and adults, whereas none of 3,7-dimehtyl-2,6-octadienal and its similar structures displayed acaricidal toxicity. This may be due to the different influences of the molecular structure on the acaricidal and repellent activities of the compounds. Ngoh et al. [22] found that the functional groups of benzene derivatives affected the fumigant toxicity, contact toxicity, and repellent activity of five benzene derivatives (eugenol, isosafrole, methyl eugenol, isoeugenol, and safrole) against the

Table 5 Acaricidal and repellent activities of 3,7-dimethyl-2,6-octadienal and its similar structures against *H. longicornis* unfed nymphs and adults

Compounds	Life cycle	Acaricidal activity[a]				Repellent activity[b]				
		LC_{50} (mg/ml) (95% CL)	LC_{90} (mg/ml) (95% CL)	Slope ± SE	χ^2 value (df, *p*)	Treatment median (s)	Negative control median (s)	*P* value[c]	Treatment/ negative control[d]	Median difference (s)[e]
Octanal	Nymphs	> 80	–	–	–	15.0	13.5	n.s.[f]	1.1	1.5
	Adults	> 80	–	–	–	7.5	7.0	n.s	1.1	0.5
2-Octenal	Nymphs	> 80	–	–	–	23.5	12.0	< 0.05	2.0	11.5
	Adults	> 80	–	–	–	14.0	7.5	< 0.05	1.9	6.5
2,4-Octadienal	Nymphs	> 80	–	–	–	28.0	13.0	< 0.05	2.2	15.0
	Adults	> 80	–	–	–	16.0	7.0	< 0.05	2.3	9.0
3,7-Dimethyl-6-octenal	Nymphs	> 80	–	–	–	71.5	12.0	< 0.01	6.0	59.5
	Adults	> 80	–	–	–	42.5	8.0	< 0.01	5.3	34.5
3,7-Dimethyl-2,6-octadienal	Nymphs	32.58 (27.75–37.84)	67.19 (55.41–88.11)	4.08 ± 0.56	6.162 (6, 0.405)	160.5	16.0	< 0.01	10.0	144.5
	Adults	41.06 (35.79–46.10)	71.27 (61.39–91.18)	5.35 ± 0.85	3.392 (6, 0.758)	96.0	9.5	< 0.01	10.1	86.5

[a] LC_{50} (LC_{90}) is the average of three determinations, with 20 ticks per replication, exposed for 24 h

[b] Dose at 0.08 mg/cm^2 of each sample

[c] Statistical significance of the difference between the treatment and negative control medians

[d] Treatment median (s)/negative control median (s)

[e] Median difference between treatment and negative control medians (s)

[f] No statistically significant difference

American cockroach, *Periplaneta americana,* supporting our findings. Based on the repellent activity of 3,7-dimethyl-2,6-octadienal and its derivatives, the unsaturated aldehydes with one or two carbon–carbon double bonds (2-octenal, 2,4-octadienal, 3,7-dimethyl-6-octenal, and 3,7-dimethyl-2,6-octadienal) exhibited repellency to the *H. longicornis* unfed nymphs and adults, whereas the saturated aldehydes with no carbon–carbon double bond (octanal) were ineffective. Furthermore, among the four unsaturated aldehydes tested, 3,7-dimethyl-2,6-octadienal with two carbon–carbon double bonds and methyl groups exhibited the highest repellency. Similar results were obtained by Hieu et al. [23]. They observed that unsaturated fatty acids (linolenic acid, linoleic acid, and oleic acid) were repellent to the stable fly, *Stomoxys calcitrans*, while saturated fatty acids (stearic acid and palmitic acid) were ineffective. The authors also demonstrated that methylated fatty acids (methyl oleate and methyl linoleate) were more repellent against *S. calcitrans* than ethyl oleate and ethyl linoleate fatty acids. Moreover, Fabbro and Nazzi [15] reported that 1-methoxy-3-vinyl-benzene (unsaturated vinyl side-chain) was more repellent to *Ixodes ricinus*. than 1-ethyl-3-methoxybenzene (saturated ethyl side-chain). Our findings support the strong influence of carbon–carbon double bonds and the functional group (methyl group) on the repellent activity of compounds against *H. longicornis,* which has previously been observed in several other arthropod species.

Previous studies have shown that 3,7-dimethyl-2,6-octadienal possesses a number of pharmacological properties, including antispasmodic [24], anxiolytic [25], and sedative properties [25]. 3,7-dimethyl-2,6-octadienal has also been listed as generally recognized as safe (GRAS) compound by the Food and Drug Administration (FDA). The acute oral LD_{50} value of 3,7-dimethyl-2,6-octadienal in rats is 6,800 mg/kg, and the dermal LD_{50} value of 3,7-dimethyl-2,6-octadienal in rats exceeds 2,000 mg/kg [26]. These results indicate that 3,7-dimethyl-2,6-octadienal has a relatively low acute toxicity in mammals.

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Authors' contributions
JHP carried out the experiments, prepared most of the data, and wrote the paper; HSL proposed the key idea of this paper, designed the experiments, managed the research process, and wrote the paper; NC designed the experiments, statistical analysis, and rewrote the paper. All authors read and approved the final manuscript.

Declarations

Competing interests
The authors declare no competing interests.

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