



Screening of essential oils with acaricidal activity against *Haemaphysalis longicornis* (Acari: Ixodidae) and analysis of active components

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ABSTRACT

Haemaphysalis longicornis (Acari: Ixodidae) is an important vector of numerous pathogens and poses a great threat to veterinary and public health. Commercially available tick repellents are extensively used and primarily comprise synthetic molecules; however, there are concerns over their safety and environmental impacts. Biologically based acaricides, particularly the plant-derived essential oils (EOs), may constitute an appealing alternative. We screened 20 different EOs by packet tests of unfed *H. longicornis* nymphs, and found that EOs of cinnamon, clove and chamomile were the most toxic (mortality > 80 %). Cinnamon EO had the most competitive acaricidal activity, with lethal concentration 50 (LC₅₀) rates of 0.4530 %, 0.2316 % and 0.0342 % (v/v) for unfed adults, nymphs and larvae, respectively. Furthermore, 5.00 % (v/v) cinnamon EO showed reproductive inhibition against *H. longicornis*, with significantly higher rates of oviposition reduction (53.19 %) and hatching reduction (46.21 %) compared with the negative control group. Composition analysis of cinnamon EO by gas chromatography–mass spectrometry (GC-MS) revealed that the major chemical compounds were trans-cinnamaldehyde (72.21 %) and cinnamic acid (19.45 %), with the former showing similar levels of acaricidal activity and oviposition inhibition as cinnamon EO. This study has demonstrated the potential of cinnamon EO and trans-cinnamaldehyde as natural acaricides against *H. longicornis*, and is the first to characterize their oviposition inhibition activity.

1. Introduction

Ticks represent an important class of ectoparasites that can cause irritation, annoyance, dermal infections, tick fever, anemia, and several distressing ailments in animals, challenging the well-being of livestock and the livestock economy worldwide (Abbas et al., 2018). Ticks are blood-sucking arachnids that feed on the host blood, influencing the defence mechanisms of the host physiologically with their salivary constituents (Cabezas-Cruz et al., 2014). Owing to their capacity to transmit various disease-causing microorganisms such as viruses, bacteria, protozoans, helminths, protists, and fungi, their medical and epidemiological impact is of paramount importance (Dantas-Torres et al., 2010). *Haemaphysalis longicornis* (Acari: Ixodidae) is native to eastern China, Korea, Japan and the Russian Far East. In addition to

being an agent of animal diseases, *H. longicornis* is an important vector of human infected diseases (Stanley et al., 2020). So far, at least 15 *H. longicornis*-associated agents have been discovered in China, of which 10 have been identified to cause disease in humans (Fang et al., 2015). For instance, the severe fever with thrombocytopenia syndrome virus (SFTSV), which causes hemorrhagic fever in humans, and the causative agent of Japanese spotted fever, *Rickettsia japonica* (Leggiadro, 2019), are both transmitted by *H. longicornis* (Luo et al., 2015) and are potentially lethal in humans (Wormser et al., 2020). In some areas of Australia and New Zealand, this tick has been reported to reduce dairy production by up to 25% in cattle (Heath, 2016). Consequently, the US Centers for Disease Control and Prevention has declared *H. longicornis* to be an emerging threat (Tanne, 2018).

The commercial practice of continual administration of biochemical

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acaricides has become the primary approach to tick control in cattle; however, it is frequently accompanied by the development of acaricide-resistant ticks, environmental pollution, and adulteration of milk and meat products with pesticide residues (Jyoti et al., 2019). Those tasked with insect pest management globally must now face the ecologic and economic fallout of the human and environmental hazards triggered by conventionally prepared pesticide compounds. Applications of botanicals used against biting insects, first documented by the early Greeks and now known as bio-acaricides, are still in practice by many societies. Recently, the development of acaricidal mixtures from botanical sources has garnered a great deal of attention, mainly due to their extreme practicability and environmentally friendly behavior against many arthropods (George et al., 2000).

Organic acaricide comprise a group of naturally occurring, mostly slow-performing defensive mediators that are generally harmless to humans, and have nominal residual effects on the environment compared with synthetic acaricides. Organic repellents, particularly plant-derived essential oils (EOs), may be a promising alternative (Benelli et al., 2016; Bissinger and Roe, 2010; Del Fabbro and Nazzi, 2013; Soutar et al., 2019). In this study, we screened 20 EOs with rich pharmacologic activities and found that cinnamon EO had the strongest acaricidal activity against *H. longicornis*. And then we characterized the composition and acaricidal properties of cinnamon EO against *H. longicornis*, supplementing the existing data in support of its use as a broad-spectrum acaricide.

2. Materials and methods

2.1. Essential oil and sample preparation

EOs (Cinnamon, Clove, Chamomile, Cumin, Tea tree, Thyme, Inula Salsoloides, Chili, Eucalyptus Leaves, Ylang-ylang, Geranium, Citronella, Rhodiola, Rose, Galangal, Patchouli, Basil, lavender, Moxa leaf, Spikenard) were purchased from ShenZhen Guoxin Essential Oil Co., Ltd. (Guangdong, China), the trans-cinnamaldehyde and cinnamic acid were purchased from Merck & Co Inc (America). The EOs and trans-cinnamaldehyde were prepared by dissolving the desired quantity in an aqueous solution containing 2.0% Tween 80 (v/v) to obtain a graded series of concentrations: 5.00%, 2.50%, 1.25%, 0.63%, 0.31% and 0.16% v/v, the cinnamic acid was prepared with 2.0% Tween 80 (v/v) at concentration of 50.0–10.0 mg/mL. It was previously determined that Tween 80 diluent alone did not cause mortality of ticks at any stage of life. Ticks in the positive control group were treated with 0.20% (v/v) cypermethrin and those in the blank control group treated with 2.0% Tween 80. Both solutions were mixed by swirling thoroughly for 1 min before use.

2.2. Gas chromatography–mass spectrometry (GC-MS) analysis of cinnamon EO

Cinnamon EO was quantitatively and qualitatively analyzed using a GC-TQ 7890–7000D system (Agilent Technologies, Palo Alto, CA, USA) equipped with an HP-5MS fused silica capillary column (30-m × 0.25-mm internal diameter; 0.25- μ m film thickness). For GC-MS detection, an electron ionization system with an ionization energy of 70 eV was used. Helium was used as the carrier gas, with a constant flow rate of 1 mL/minute. Injector and mass transfer line temperatures were set at 250 °C and 280 °C, respectively. EO solution (1 μ L) in hexane was injected and analyzed under the following column conditions: initial column temperature of 40 °C for 1 min, which was increased to 250 °C with a 3 °C/minute heating ramp, then maintained at 250 °C for 20 min. Kovats indices were calculated for all volatile components using a homologous series of n-alkanes (C8–C25) in an HP-5 MS column. The major oil components were identified by co-injection with standards (whenever possible) and confirmed using Kovats indices from the National Institute of Standards and Technology 17 Mass Spectral Libraries.

The relative concentrations of each compound in the oil were quantified based on the peak area integrated in the analysis program (Alizadeh Behbahani et al., 2020).

2.3. Ticks

Approximately 200 adult male and female *H. longicornis* specimens were collected from the Xiaowutai National Natural Reserve Area (114°47'–115°28' E, 39°50'–40°6' N) of Hebei Province, China. No acaricides had been applied to this tick population prior to collection. The ticks were identified morphologically according to characteristics described by Teng and Jiang (1991), then transported back to the laboratory. One group of ticks was incubated in gauze-plugged tubes, which were placed in an environmental chamber at $80 \pm 5\%$ RH, 28 ± 1 °C and a 14-hour light/10-hour dark cycle (Liu et al., 2005). A second group was reared on domestic breeding rabbits in the GLP Center of Lanzhou Institute of Husbandry and Pharmaceutical Science of the Chinese Academy of Agricultural Sciences. The rabbits were maintained at 20–25 °C room temperature and 50% relative humidity (RH), under natural daylight cycles, and were infested with the ticks on one occasion. Eggs and ticks at all stages were obtained from both groups.

2.4. Packet test for larvae and nymphs

The packet test was carried out as described by Stone and Haydock (1962), with adaptations (Monteiro et al., 2012). Briefly, about 100 unfed larvae or 100 nymphs (age, 10 days) were picked for each treatment group, and individually transferred using a paintbrush to the center of a sheet of filter paper measuring 6×6 cm, which was then folded and closed at the edges with clips to form a packet. Next, each packet containing ticks was evenly moistened with 1 mL EO (trans-cinnamaldehyde or cinnamic acid) solution at each concentration to be tested. The negative control group was treated with 2% Tween 80, and the positive control group with 0.20% v/v cypermethrin. Each treatment group was tested in three replicates. The packets were placed in the environmental chamber at 25 ± 2 °C and $80 \pm 5\%$ RH, opened at 3, 6, 9, 12, 24 and 48 h of incubation and observed under a microscope (BX43, Olympus). The numbers of living and dead larvae were counted manually, and only ticks capable of locomotion were considered to be alive. In the preliminary screening test of 20 essential oils, approximately 24–25 unfed *H. longicornis* nymphs from each group, using a 5.00% concentration of each EO, and were repeated three times for each oil, the operation method is consistent with the above mentioned.

2.5. Immersion test for unfed adults

The immersion test was carried out as described by Drummond et al. (1973) with minor modifications. Briefly, approximately 720 unfed adults (age, 2–3 weeks) were separated and randomly divided into 20 ticks per group. For each treatment group, 20 unfed adults were placed into centrifuge tubes, and immersed for 5 min in 6-mL solutions of different concentrations of EOs (trans-cinnamaldehyde or cinnamic acid) with enough gentle shaking to cause all of the ticks to soak below the liquid level. The negative control group was immersed in 2% Tween 80 and the positive group in 0.20% v/v cypermethrin. Each treatment group was tested in three replicates. After soaking, the ticks were removed from the solution, cleaned of any residual liquid on the body using filter paper, placed into a clean 5-mL test tube, and stored in the environmental chamber at 25 ± 2 °C and $80 \pm 5\%$ RH. The ticks were evaluated for mortality 48 h later with the aid of the microscope; ticks that could not move their appendages even when pressed hard with tweezers were considered dead. The average tick mortality rate was calculated and corrected using Abbott's (1925) formula:

$$\text{Corrected mortality} = \frac{\% \text{Mortality in test group} - \% \text{Mortality in control group}}{100 - \% \text{Mortality in control group}} \times 100$$

2.6. Immersion test for engorged adults

The test was performed as described previously (Drummond et al., 1973), with minor modifications, on engorged female adult ticks. The engorged female ticks were weighed and randomly separated into groups containing 10 ticks each. The groups were immersed in 40-mL solutions of different concentrations of EOs (trans-cinnamaldehyde or cinnamic acid) in a 50-mL beaker, which was gently agitated (three times) at room temperature. After exactly 5 min of immersion, the solution was poured off through a sieve, and the ticks were transferred into Petri dishes and kept in incubators at a controlled temperature of 25

$\pm 2^\circ\text{C}$ and $80 \pm 5\%$ RH. The negative control group was immersed in 2% Tween 80. After incubating the ticks for 2–3 weeks, the mortality rates were calculated and the eggs produced by each group were weighed. The eggs were transferred to 5-mL plastic syringes and placed back into the incubator for larval hatching under the same conditions. After 2–3 weeks, the syringes were observed, and the hatching rates for the different treatments were estimated and compared with the control group. All treatment groups and the control were set up in five replicates for each concentration tested. The data obtained were used to calculate the percent reduction of oviposition (% OR) and hatching (% HR) as described by Gupta et al., (2016), as well as the estimated reproduction (ER), which was used to calculate the treatment efficacy (E) (Drummond et al., 1973) as follows:

$$\text{OR} = \frac{\text{mean weight of eggs in control(g)} - \text{weight of eggs in treated group(g)}}{\text{mean weight of eggs in control (g)}} \times 100$$

$$\text{HR} = \frac{\text{mean hatching rate in control} - \text{hatching rate in treated group}}{\text{hatching rate in control}} \times 100$$

$$\text{ER} = \frac{\text{weight of eggs(g)} \times \% \text{hatching rate}}{\text{weight of females(g)}}$$

$$E = \frac{\text{ER control} - \text{ER treated}}{\text{ER control}} \times 100$$

2.7. Statistical analysis

The larval mortality data were subjected to Probit analysis (SPSS 26) for calculations of lethal dose (LC_{50} , LC_5 and LC_{95}), and other statistics at 95% fiducial limits of upper confidence and lower confidence. The significance of mean differences between groups was statistically compared using analysis of variance (ANOVA) at the 5% probability level, with ANOVA comparisons made using Duncan's test (SPSS 26).

3. Results

3.1. Screening of potential acaricidal EOs against *H. longicornis*

Twenty EOs described in the literature were tested for acaricidal effects on *H. longicornis*. The results showed that compared with other 17 essential oils, cinnamon EO, clove EO and chamomile EO had obvious toxic effects against the nymphs, with mortality rates of 100%, 100% and 89.3%, respectively, at 24 h (Table 1). The mortality rates of the other oils were all below 80%. Therefore, these three EOs were considered to have relatively good killing potential against *H. longicornis* and were used for the subsequent experiments.

Table 1

Acaricidal activity of 20 essential oils on unfed nymphs of *H. longicornis* (each experiment was repeated three times).

Name	Plants	Concentration (v/v)	Mortality (%)
Cinnamon	<i>Cinnamomum cassia</i> L.	5.00%	100.0 \pm 0.0
Clove	<i>Syzygium aromaticum</i> L.	5.00%	100.0 \pm 0.0
Chamomile	<i>Matricaria chamomilla</i> L.	5.00%	89.3 \pm 4.6
Cumin	<i>Foeniculi fructus</i>	5.00%	74.4 \pm 8.0
Tea tree	<i>Melaleuca alternifolia</i>	5.00%	73.0 \pm 8.2
Thyme	<i>Thymus mongolicus</i>	5.00%	69.0 \pm 7.9
Inula Salsoloides	<i>Inula salsoloides</i>	5.00%	67.7 \pm 10.1
Chili	<i>Capsicum annum</i> L.	5.00%	67.6 \pm 6.6
Eucalyptus Leaves	<i>folium eucalypti</i>	5.00%	66.2 \pm 10.0
Ylang-ylang	<i>Cananga odorata</i>	5.00%	50.1 \pm 5.4
Geranium	<i>Pelargonium graveolens</i>	5.00%	47.4 \pm 9.6
Citronella	<i>Cymbopogon winteratus</i>	5.00%	47.3 \pm 10.3
Rhodiola	<i>Rhodiola rosea</i> L.	5.00%	40.7 \pm 11.4
Rose	<i>Rosa centifolia</i> L.	5.00%	39.1 \pm 7.8
Galangal	<i>Kaempferiae Parviflorae</i>	5.00%	37.8 \pm 2.0
Patchouli	<i>Pogostemon cablin</i>	5.00%	37.8 \pm 8.9
Basil	<i>Ocimum basilicum</i>	5.00%	36.4 \pm 3.4
lavender	<i>Lavandula angustifolia</i>	5.00%	35.1 \pm 7.9
Moxa leaf	<i>Folium Artemisiae Argyi</i>	5.00%	31.1 \pm 6.3
Spikenard	<i>Nardostachys jatamansi</i> DC.	5.00%	27.1 \pm 6.3

3.2. Acaricidal activity of three EOs on different growth stages of *H. longicornis*

Fig. 1 A shows the killing effects of the EOs on unfed larvae presents increasing trends with concentration and time. Cinnamon and clove EOs each had a 100% killing effect on *H. longicornis* unfed larvae after 3 h at concentrations of 0.63%, but the acaricidal effects of clove EO decreased significantly below 0.63%. By contrast, a concentration of 2.5% chamomile EO was required to achieve a 100% killing effect, and a concentration of 0.63% produced almost no acaricidal effect. These results indicated that cinnamon and clove were more toxic than chamomile against *H. longicornis* unfed larvae, and that cinnamon EO was superior to clove EO. Furthermore, Table 2 shows that the LC_{50} of cinnamon EO on larvae (0.0342% v/v) at 24 h was significantly lower than those for clove EO (0.1662% v/v) and chamomile EO (0.7597% v/v).

The results in nymphs were similar to those in larvae. Cinnamon and clove EOs reached a 100% killing effect at 2.5% concentration, among the three, cinnamon EO having relatively stronger effect (Fig. 1B). The LC_{50} of cinnamon EO on nymphs (0.2316%) was lower than those for clove EO (0.4847%) and chamomile EO (0.9594%) (Table 2). The immersion test for engorged female adults showed that cinnamon was the only EO with lethal action at a concentration of < 5.00% (Fig. 2), the insecticidal effects of clove and chamomile EOs were very weak at this concentration. Therefore, of the three EOs tested, cinnamon EO had the better acaricidal activity at different growth stages of *H. longicornis*.

3.3. Effects of three EOs on the reproductive capacity of *H. longicornis*

Table 3 shows the reproductive capacity of engorged female adults following immersion in different concentrations of EOs. Whereas the lethality rate of immersion in 5.00% (v/v) cypermethrin was 100%, there was no apparent lethality of 5.00% clove or chamomile EOs or the 2% Tween 80 control. A few ticks died following immersion in 5.00% cinnamon EO. Exposure to 5% concentrations of cinnamon, clove and chamomile EOs also resulted in oviposition reductions (ORs) of 53.19%, 46.21% and 38.49%, hatching reductions (HRs) of 50.0%, 43.39% and 33.08%, and final efficacy rates of 76.95%, 69.99% and 69.35%, respectively. These results were significantly higher ($P < 0.05$) than those obtained in the 2% Tween 80 control group. The OR and HR percentages increased with increasing concentrations of all three EOs, with cinnamon EO showing a relatively more significant growth trend.

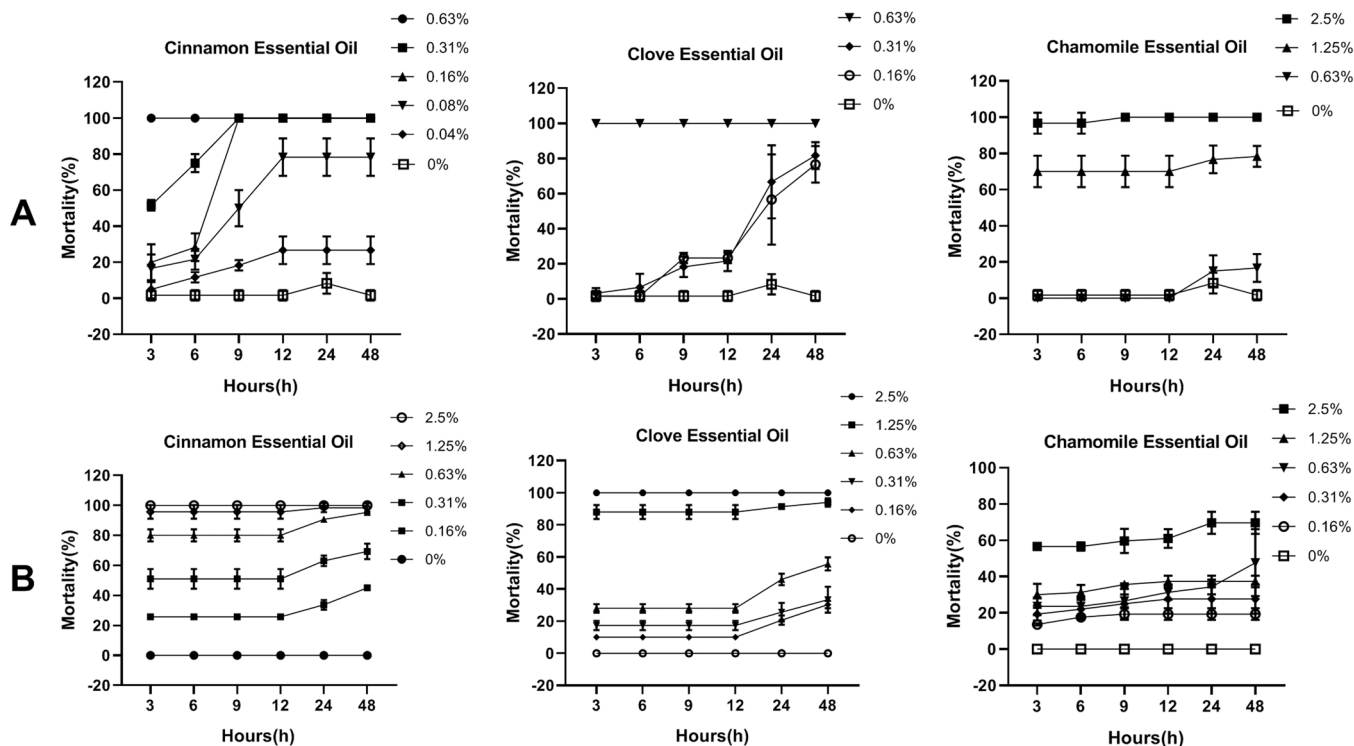


Fig. 1. Mortality evaluation of acaricidal activities of essential oils of cinnamon, clove and chamomile against (A) unfed larvae and (B) unfed nymphs of *Haemaphysalis longicornis* at 3, 6, 9, 12, 24 and 48 h after treatment.

Table 2

Lethal concentration of 50% (LC₅₀) and 95% confidence interval (95% CI) of three essential oil for different growth stages of *H. longicornis* at 24 h (each experiment was repeated three times).

	Essential Oil	LC ₅₀ (% v/v)	LC ₅₀ CI	LC ₅ (% v/v)	LC ₉₅ (% v/v)
Adult	Cinnamon	0.4530	0.2831–0.6733	0.1019	2.014
	Cinnamon	0.2316	0.1634–0.2958	0.0691	0.7753
	Clove	0.4847	0.3662–0.6259	0.1174	2.001
Larvae	Chamomile	0.9594	0.6508–1.4368	0.0665	13.83
	Cinnamon	0.0342	0.0245–0.0443	0.0095	0.1229
	Clove	0.1662	0.0897–0.2248	0.0460	0.6001
	Chamomile	0.7597	0.5869–0.9802	0.2027	2.8475

When the concentration of cinnamon EO was decreased to 2.5% (v/v), the rates of OR and HR remained high at 45.52% and 46.70%, respectively, and were still at 16.40% and 8.37%, respectively, at the 0.16% (v/v) concentration. Cypermethrin was the only treatment that produced 100% rates of OR and HR at the 0.20% w/v concentration.

3.4. Constituent analysis of cinnamon EO

Considering its preferable acaricidal and oviposition inhibition activities at different stages of *H. longicornis* compared with other two EOs, cinnamon EO was chosen for active ingredient analysis. The cinnamon EO was pale yellow in color and showed the following results on GC-MS analysis. Twenty compounds comprising 99.98% of the total were identified (Table 4). The two major chemical compounds were trans-cinnamaldehyde (72.21%) and cinnamic acid (19.45%). Trans-cinnamaldehyde was obtained as a light yellow oily liquid, with a melting point of -7.5 °C, boiling point of 127 °C (2.13 kPa), and relative density of 1.0497 (20/4 °C). It was soluble in alcohol and chloroform, and slightly soluble in water. Trans-cinnamaldehyde has the signature aroma of cinnamon and well-established antibacterial and antifungal effects; more recently, there have been reports of its insecticidal effects (Nwanade et al., 2021). Trans-cinnamaldehyde is the main component (55–85%) of commercial cinnamon oils from China and Ceylon (Sri Lanka), and is found in the trans-structured form in both natural and synthetic products (Doylea et al., 2019).

3.5. Acaricidal and anti-reproductive effects of trans-cinnamaldehyde on *H. longicornis*

As the main components of cinnamon EO, trans-cinnamaldehyde and cinnamic acid were also tested for their acaricidal effects on *H. longicornis*. Trans-cinnamaldehyde had a similar killing effect as cinnamon EO: at a concentration of 0.31%, a 100% tick-killing effect on unfed larval was achieved in 3 h (Fig. 3); 0.63% concentration of cinnamon EO was required for the same effect (Fig. 1). For unfed adults,

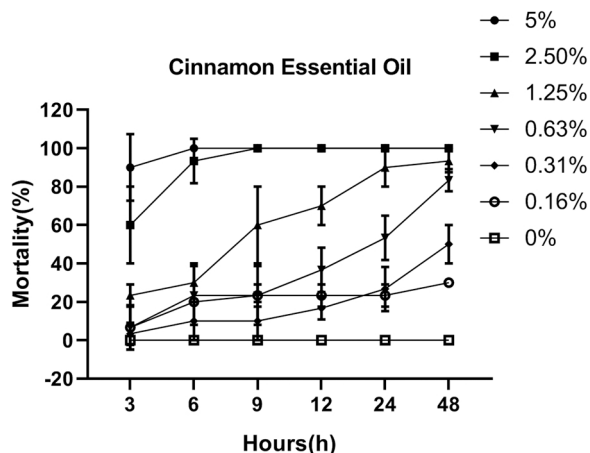


Fig. 2. Mortality evaluation of acaricidal activity of cinnamon essential oil against unfed adults of *Haemaphysalis longicornis* at 3, 6, 9, 12, 24 and 48 h after treatment.

Table 3

Oviposition reduction (OR), hatching reduction (HR), estimated reproduction (ER), and efficacy (E) of *H. longicornis* engorged female adults after immersion in different dilutions of three essential oils.

Sample	Concentration (% v/v)	OR (%)	HR (%)	ER	E (%)
Cinnamon essential oil	0.16	16.40 ± 6.43 ^d	8.37 ± 3.15 ^f	39.27 ± 15.97 ^a	6.63 ± 0.19 ^d
	0.31	18.22 ± 5.46 ^d	20.04 ± 9.52 ^e	28.84 ± 5.83 ^b	27.34 ± 14.68 ^d
	0.63	22.77 ± 7.56 ^d	30.84 ± 11.56 ^d	20.68 ± 6.04 ^c	47.91 ± 15.22 ^c
	1.25	40.64 ± 8.02 ^c	39.21 ± 6.63 ^{c,d}	13.32 ± 1.90 ^{c,d}	66.44 ± 4.79 ^b
	2.50	45.52 ± 9.74 ^{b,c}	46.70 ± 7.37 ^{b,c}	9.58 ± 4.35 ^d	75.86 ± 10.97 ^b
5.00	53.19 ± 5.29 ^b	50.00 ± 5.75 ^b	9.15 ± 2.61 ^d	76.95 ± 6.57 ^b	
Tween 80	2.0	0.0 ± 1.3 ^e	0.0 ± 4.9 ^f	39.69 ± 9.49 ^a	0.0 ± 3.0 ^e
Cypermethrin	0.20	100 ± 0.0 ^a	100 ± 0.0 ^a	0.0 ± 0.0 ^e	100 ± 0.0 ^a
Clove essential oil	0.16	11.86 ± 3.76 ^{d,e}	6.17 ± 4.08 ^f	40.46 ± 15.85 ^a	4.82 ± 1.38 ^{e,f}
	0.31	14.18 ± 3.61 ^d	16.52 ± 4.57 ^e	34.50 ± 7.42 ^a	13.08 ± 5.69 ^e
	0.63	17.93 ± 7.95 ^d	23.79 ± 6.49 ^d	22.12 ± 5.89 ^b	44.26 ± 14.83 ^d
	1.25	33.02 ± 4.72 ^c	27.75 ± 5.81 ^{c,d}	18.20 ± 5.15 ^{b,c}	54.14 ± 12.97 ^{c,d}
	2.50	38.91 ± 7.89 ^{b,c}	34.36 ± 9.03 ^c	12.57 ± 4.32 ^c	68.33 ± 10.89 ^{b,c}
5.00	46.21 ± 10.98 ^b	43.39 ± 5.81 ^b	11.91 ± 4.66 ^c	69.99 ± 11.74 ^b	
Tween 80	2.0	0.0 ± 1.3 ^e	0.0 ± 4.9 ^f	39.69 ± 9.49 ^a	0.0 ± 3.0 ^f
Cypermethrin	0.20	100 ± 0.0 ^a	100 ± 0.0 ^a	0.0 ± 0.0 ^d	100 ± 0.0 ^a
Chamomile essential oil	0.16	7.55 ± 1.78 ^e	2.66 ± 1.76 ^{f,g}	45.03 ± 10.40 ^a	5.51 ± 0.60 ^{d,e}
	0.31	8.91 ± 3.37 ^e	6.17 ± 2.76 ^f	35.05 ± 7.58 ^{b,c}	19.10 ± 5.69 ^{d,e}
	0.63	11.23 ± 4.58 ^e	14.10 ± 3.73 ^e	30.79 ± 8.31 ^{c,d}	22.44 ± 6.93 ^d
	1.25	17.71 ± 4.70 ^d	20.26 ± 3.36 ^d	23.87 ± 5.14 ^d	39.87 ± 12.95 ^c
	2.50	24.51 ± 4.78 ^c	27.31 ± 6.88 ^c	14.37 ± 5.43 ^e	63.80 ± 13.69 ^b
5.00	38.49 ± 8.08 ^b	33.08 ± 5.08 ^b	7.17 ± 3.75 ^e	69.35 ± 9.45 ^b	
Tween 80	2.0	0.0 ± 1.3 ^f	0.0 ± 4.9 ^g	39.69 ± 9.49 ^{a,b}	0.0 ± 3.0 ^e
Cypermethrin	0.20	100 ± 0.0 ^a	100 ± 0.0 ^a	0.0 ± 0.0 ^f	100 ± 0.0 ^a

Values in the same column with different subscript are significantly different; analyzed by Duncan's test at 5% probability ($p < 0.05$).

Table 4

Chemical composition of cinnamon essential oil.

Peak	RI ^a	Components	%RA ^b	CAS	Identification methods ^c
1	962	Benzaldehyde	3.03	100–52–7	MS, RI
2	1065	Acetophenone	0.06	98–86–2	MS, RI
3	1066	Ethanone, 2,2-dihydroxy-1-phenyl-	0.16	1075–06–5	MS, RI
4	1170	Benzoic acid	0.38	65–85–0	MS, RI
5	1387	3,4-Dihydrocoumarin	2.66	119–84–6	MS, RI
6	1175	1,2-Propanedione,1-phenyl-	0.1	5651–24–1	MS, RI
7	1270	Trans-Cinnamaldehyde	71.82	14371–10–9	MS, RI, Co
8	1309	-Propenal,2-methyl-3-(4-methylphenyl)	0.06	3893–15–0	MS, RI
9	1451	trans-Cinnamic acid	0.13	140–10–3	MS, RI
10	1402	Methyleugenol	0.1	31706–95–3	MS, RI
11	1435	3-Phenylpropyl cinnamate	19.78	122–68–9	MS, RI, Co
12	1464	1-Hydroxy-1,2,3,4-tetrahydronaphthalene-8-carboxaldehyde	0.2	78323–74–7	MS, RI
13	1483	Cyclobutanol	0.06	20117–47–9	MS, RI
14	1490	2,4,6-Cycloheptatrien-1-one	0.08	539–80–0	MS, RI
15	1500	Stilbene	0.06	103–30–0	MS, RI
16	1971	trans-Chalcone	0.06	614–47–1	MS, RI
17	1998	Furan	0.26	110–00–9	MS, RI
18	2094	1,4-Butanedione	0.45	1107–23–9	MS, RI
19	2147	3-cyclopenten-1-one	0.5	6947–63–3	MS, RI
20	2250	Tributyl acetyl citrate	0.034	77–90–7	MS, RI

^a Retention index relative to n-alkanes on HP-5 MS capillary column.

^b Relative area (peak area relative to the total peak area).

^c RI, retention index; MS, mass spectrum; Co, co-injection with authentic compound.

concentrations of 2.5% trans-cinnamaldehyde and 5.00% cinnamon EO were required to achieve 100% mortality in a short period of time. The acaricidal activities of trans-cinnamaldehyde and cinnamon EO were nearly similar in unfed larvae and nymphs (Figs. 2 and 3). The results of immersion tests of engorged adults in trans-cinnamaldehyde were basically the same as those for cinnamon EO: at 5.00% (v/v), there was 58.64% OR, 52.32% HR, and 76.42% E. These results were significantly higher ($P < 0.05$) than those obtained in the 2% Tween 80 control group (Table 5). By contrast, cinnamic acid showed almost no tick-killing activity at any of the three growth stages, and had no effect on the reproductive capacity of *H. longicornis*.

4. Discussion

Parasitic diseases are an important health hazard in humans and animals in developing countries, and the ectoparasite *H. longicornis* has become a serious threat to public health and economies all around the world. Ticks cause annoyance, irritation, skin infections, anemia and tick fever, and spread devastating vector-borne illnesses in livestock, pets and humans. Over the last 50 years, ticks have mainly been controlled with synthetic insecticides. However, the indiscriminate use of chemical pesticides has given rise to many serious problems, including genetic resistance of pest species, toxic residues in stored products, increasing costs of application, handling hazards, and negative non-target effects on humans and other organisms. Although the recognition of chemical pesticides as environmental pollutants should

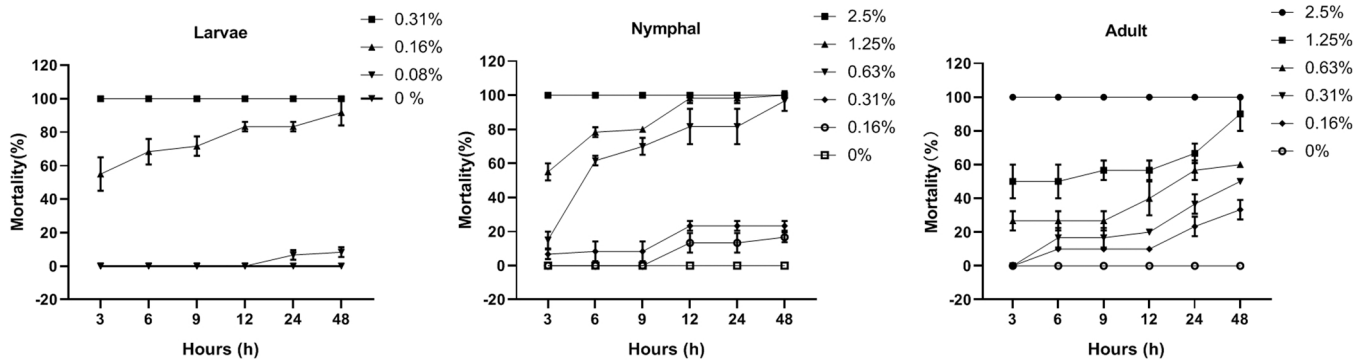


Fig. 3. Mortality evaluation of acaricidal activity of trans-cinnamaldehyde against unfed larvae, nymphs and adults of *Haemaphysalis longicornis* at 3, 6, 9, 12, 24 and 48 h after treatment.

Table 5

Oviposition reduction (OR), hatching reduction (HR), estimated reproduction (ER), and efficacy (E) of *Haemaphysalis longicornis* engorged female adults after immersion in trans-cinnamaldehyde.

Sample	Concentration (% v/v)	OR (%)	HR (%)	ER	E (%)
Trans-cinnamaldehyde	0.16	20.40 ± 8.67 ^c	9.05 ± 4.22 ^c	45.00 ± 14.39 ^a	10.37 ± 4.03 ^c
	0.31	21.22 ± 6.09 ^{d,e}	15.62 ± 6.26 ^d	34.97 ± 9.74 ^b	30.82 ± 9.34 ^d
	0.63	30.77 ± 10.21 ^d	27.04 ± 17.93 ^c	26.74 ± 10.87 ^{b,c}	49.00 ± 18.24 ^c
	1.25	43.64 ± 12.46 ^c	36.73 ± 10.35 ^b	15.38 ± 3.56 ^{c,d}	68.97 ± 21.38 ^{b,c}
	2.50	49.52 ± 11.60 ^c	49.76 ± 13.49 ^b	11.30 ± 5.04 ^d	74.20 ± 25.03 ^b
Tween 80	2.0	58.64 ± 21.05 ^b	52.32 ± 19.75 ^b	9.73 ± 3.09 ^d	76.42 ± 15.98 ^b
Cypermethrin	0.20	0.0 ± 1.3 ^f	0.0 ± 4.9 ^f	39.69 ± 9.49 ^a	0.0 ± 3.0 ^f
		100 ± 0.0 ^a	100 ± 0.0 ^a	0.0 ± 0.0 ^e	100 ± 0.0 ^a

Values in the same column with different subscript are significantly different; analyzed by Duncan's test at 5% probability ($p < 0.05$).

not detract from their importance in agriculture, animal husbandry and public health, biologically-based acaricides are associated with lower risks to the environment and mammals, higher specificity and safety to non-target organisms, lower risks of resistance development, and lower environmental persistence.

Plant-derived EOs may constitute an especially appealing alternative. The use of herbal extracts or their EOs in the control of ectoparasites can reduce costs and environmental impacts. EOs are natural compounds that have volatile molecule components with strong odors and are generally soluble in organic solvents. Botanical EOs are complex mixtures of 20–60 low-molecular-weight metabolites produced by aromatic plants, and are typically characterized by two or three major terpene or terpenoid components, which constitute up to 30% of the oil. In recent years, some plant EOs have been found to have a repellent effect against ticks. Such as EOs of *Amyris balsamifera* (Rutaceae) and Osage orange were shown to have repellent activity against nymphs of the blacklegged tick, *Ixodes scapularis*, and the lone star tick, *Amblyomma americanum* (Carroll et al., 2010). The Libyan plants *Salvadora persica* and *Rosmarinus officinalis* were shown to have EOs with repellent and toxic effects against nymphs of *Ixodes ricinus* (Elmhalli et al., 2019). Also, the geranium EO has been reported to have good repelling properties on ticks including *Ixodes ricinus* L. and *Amblyomma americanum* (L.) (Tabanca et al., 2013). Similarly, more and more studies have reported acaricidal activities of plant EOs and their active ingredients against a variety of ticks. The EO extract from *Schinus molle* L. had acaricidal effects on unfed larvae and a significant inhibitory effect on the reproductive ability of *Rhipicephalus sanguineus* (Rey-Valeiron et al., 2018). Jia et al. (2018) reported that *Arisaema anurans* EO and its major constituents had toxicity against *Rhipicephalus microplus*. EOs obtained from *Juniperus thurifera* var. *africana* and *Mentha suaveolens* subsp. *Timija* chemotypes showed acaricidal activity against pre-adult stages of *Hyalomma aegyptium* ticks (El-Mustapha et al., 2021). The Cumin EO has been reported had acaricidal activity against cattle tick *Rhipicephalus microplus* (Estefania et al., 2019), and the Tea Tree EO also has toxicity against cattle tick larvae (Yim et al., 2016). In the experiments on

free-ranging Boran cattle showed that citronella EO had a good insecticidal effect on ticks (Grzeda et al., 2017). In addition, the potential acaricidal effect of galangal oil on ticks is reported in a recent study (Kang et al., 2022). Finally, acetylcarvacrol was found to be the most important ingredient of *Origanum* sp. and *Thymus* sp. in their impact on inhibition on the reproductive system of *R. microplus*, causing several types of damage to female germ cells (Konig et al., 2019). Nevertheless, the acaricidal effect of EOs against *H. longicornis* are poorly understood.

To screen for the effective EOs against *H. longicornis*, a collection of 20 commercial EOs previously reported to have insecticidal, acaricidal, antifungal, anti-inflammatory or antimicrobial activities were prepared and tested for acaricidal activity on unfed nymphs (Table 1). Although the cinnamon, clove and chamomile EOs delivered mortality rates of 100%, 100% and 89.3%, respectively, at 24 h, those for the other oils were below 80%. The ylang-ylang EO has been reported to have acaricidal activity against *Ixodes ricinus* (Elmhalli et al., 2018), however in this study its acaricidal activity was weaker than those of cinnamon, chamomile and clove against unfed *H. longicornis* nymphs at the same concentration, the mortality rate was merely 50%. Although Thyme essential oil was considered has effective repellent action against the lone star tick (Meng et al., 2016), the mortality rate was only 69% in the killing test of unfed *H. longicornis* nymphs. It was reported that an 8% concentration of EO from chamomile flowers caused 46.67% failure in egg laying in engorged females of the cattle fever tick (Pirali-Kheirabadi and Razzaghi-Abyaneh, 2007), but the killing effects at different growth stages were not mentioned. EO extracts from clove buds showed repellent efficacy against adult *Dermacentor reticulatus*, lone star and cattle ticks (Ferreira et al., 2018; Machtinger and Li, 2017; Mello et al., 2014; Stefanidesova et al., 2017). Prior to this study, the acaricidal effects of chamomile and clove EOs against *H. longicornis* were unknown, whereas the toxic effects of cinnamon EO on ticks had been examined by other researchers. Cinnamon oil was reported as having the potential to control the cattle tick (Diaz et al., 2019; Santos et al., 2017; Jyoti et al., 2019; Marchesini et al., 2021) and to repel nymphs of the lone star tick (Meng et al., 2016). Although Nwanade et al. (2021) performed a

preliminary study on the toxic effects of cinnamon EO on larvae and nymphs of *H. longicornis*, its effect on reproductive performance needed to be explored further.

Cinnamon EO is used as a natural food preservative and for flavoring, and is one of the pharmacologically active components of *Cinnamomum verum* Presl (Monteiro et al., 2017). Trans-cinnamaldehyde, a chemical component of cinnamon oil, has antiparasitic, bactericidal, animal repellent and antifungal properties (Bassole and Juliani, 2012; Cheng et al., 2006; Lirussi et al., 2004; Santos et al., 2017), and has been used in pharmacology for centuries (Antunes and Cavaco, 2010). Although the acaricidal effect of cinnamon EO against the cattle tick *Rhipicephalus microplus* (Acari: Ixodidae) has been well characterized, very few studies of which have been carried out on *H. longicornis*. In this study, we chose to examine the acaricidal activities of EOs of cinnamon, clove and chamomile against *H. longicornis*. Fig. 1 A shows that cinnamon EO achieved 100% mortality on unfed larvae at concentrations of 0.63% (v/v) at 3 h, and 0.16% at 9 h. When the concentration is as low as 0.08%, the tick-killing effect reached 80% mortality in 12 h. Although clove EO had a similar tick-killing effect as cinnamon at 0.63%, the effect decreased significantly with decreasing concentration, and chamomile EO required a 2.5% concentration to achieve the same effect as cinnamon. Tests on unfed nymphs showed trends that were similar to unfed larvae (Fig. 1B): the lethal doses of cinnamon and clove EOs were 2.5%. Thus, all three EOs had toxicity against *H. longicornis*, with cinnamon showing superior performance to clove and chamomile. The experimental results of cinnamon EO on larvae (Table 2) were similar to those in the literature, but the LC₅₀ of nymphs was lower than that reported by Nwanade et al. (2021), possibly because of differences between the studies in terms of batches and number of days post-hatching. Only cinnamon EO was lethal to unfed adult ticks (Fig. 2).

The relatively superior acaricidal activity of cinnamon EO against *H. longicornis* led us to analyze its composition by GC-MS (Table 4). Although Nwanade et al. (2021) also found that the combined content of trans-cinnamaldehyde and cinnamic acid exceeded 90% of the total, the percentage composition of trans-cinnamaldehyde was relatively lower (50.79%) in their study compared with ours (71.82%), possibly because the oils were derived from plants with different geographic origins. The toxicities of trans-cinnamaldehyde and cinnamic acid against *H. longicornis* were also measured, with trans-cinnamaldehyde showing acaricidal activity comparable to cinnamon EO (Fig. 3) and cinnamic acid showing no activity.

Cinnamon EO immersion inhibited the reproductive capacity (50% OR) of engorged female adults at approximately 2.5% concentration compared with > 5.00% required for the same effect with clove and chamomile EOs, and was lethal to some engorged female adults at 5.00% (Table 3); thus, it was the most effective of the three at inhibiting oviposition. Moreover, trans-cinnamaldehyde showed the almost same reproductive effects as cinnamon EO. Recent studies on the inhibitory effect of cinnamon EO on insect oviposition revealed that cinnamon oil and trans-cinnamaldehyde could inhibit oviposition in the box tree moth (*Cydalima perspectalis*) (Szelenyi et al., 2020), the southern house mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae) (Andrade-Ochoa et al., 2018), *Sitophilus oryzae* (Devi et al., 2013), *Anopheles stephensi* (Prajapati et al., 2005), *Drosophila suzukii* (Diptera: Drosophilidae) (Eben et al., 2020), and *Lucilia sericata* (Khatir and Geden, 2018). This is the first study to report oviposition inhibition by cinnamon EO in *H. longicornis*.

In conclusion, cinnamon EO, a common food additive with ancient origins, has the advantages of being eco-friendly, safe for non-targets, and geographically wide ranging. Trans-cinnamaldehyde, the main acaricidal component of cinnamon EO against *H. longicornis*, may have potential as an environmentally protective insecticide. Although cinnamon EO and trans-cinnamaldehyde have obvious acaricidal effect activity not only according to the literature but also in this study, they are still different from synthetic insecticides in dose. Future studies should investigate the mechanism of its insecticidal effect using

transcriptomics to examine differential gene expression in response to treatment with cinnamon EO. The insecticidal mechanism of cinnamon EO may lead researchers to explore more strategies for killing *H. longicornis*.

CRedit authorship contribution statement

Ruihua Xin: Methodology, Investigation, Data curation, Writing – original draft, Writing – review and editing. **Guibo Wang** and **Zhen-gyong Qiu:** Data curation and processing, Investigation. **Qian Ma:** Biological studies, Data curation. **Saad Ahmad:** Writing – review and editing. **Feng Yang** and **Hui Wang:** biological studies. **Jiongjie He** and **Xiaohan Jing:** Methodology. **Yan Sun:** Funding acquisition, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Animal welfare statement

Institutional ethical and animal care guidelines were observed, and all procedures were conducted according to the China Guide for the Care and Use of Laboratory Animals (protocol number: IACUC-157031).

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