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RESEARCH ARTICLE

Toxicity and Synergistic Interactions of *Citrus limon* Essential Oil and Limonene with Chemical Acaricides Against *Haemaphysalis doenitzi* (Acari: Ixodidae)

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ABSTRACT

Haemaphysalis (*H.*) *doenitzi* is a vector for the transmission of pathogens to endangered and domesticated animals, and the need for its control is becoming increasingly urgent. This study evaluated plant essential oils (EOs) and their major components as eco-friendly alternatives to chemical acaricides, including synergistic effects with acaricides. GC-MS analysis identified limonene (86.76%) and α-hexylcinnamaldehyde (21.71%) as the main components of *Citrus* (*C.*) *limon* and *Jasminum* (*J.*) *sambac* EO, respectively. Immersion tests showed both EOs are highly effective against *H. doenitzi* nymphs and adults. *Citrus limon* EO showed higher acaricidal toxicity than *J. sambac* EO, whereas the major component of *J. sambac* EO was more potent than limonene. *Citrus limon* EO and limonene inhibited CarE activity and upregulated ABC transporter expression. Molecular docking revealed limonene has a stronger binding affinity to ABC transporters, GSTs, and CYP450 enzymes. Tick control trials showed *J. sambac* EO increased engorgement weight, whereas *C. limon* EO reduced both engorgement and egg mass weight. RNAi knockdown of chitin binding protein (*HDCBPa*) and fumarylacetoacetase (*HDFAHa*) significantly increased tick sensitivity to *C. limon* EO and limonene, leading to higher mortality. Synergistically, a 1:3 mixture of *C. limon* EO and pyridaben produced a co-toxicity coefficient (CTC) of 129.71. In conclusion, *C. limon* EO and limonene may exert acaricidal effects by inhibiting CarE, upregulating ABC transporter gene, and binding enzymes, while reducing tick weight and egg mass and enhancing pyridaben efficacy, highlighting their superior potential for tick control.

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INTRODUCTION

Ticks are obligate hematophagous ectoparasites that feed on reptiles, birds, and mammals, and serve as important vectors for various pathogens, including viruses, bacteria, and protozoa (Husin *et al*., 2026; Moraga *et al*., 2023). *Haemaphysalis* (*H.*) *doenitzi* is frequently found on the body surfaces of birds (Zhang *et al*., 2024a). As a vector for babesiosis, *H. doenitzi* is also widely distributed in areas endemic for severe fever, posing a significant public health challenge (Zhang *et al*., 2025a; Wang *et al*., 2025). In recent years, climate warming, host migration, and ecological changes have altered tick population dynamics, increasing the likelihood of human and animal exposure to ticks (Bonizzoni and Lahondère, 2026). The threat of tick-borne diseases has become increasingly severe. In livestock production, serious tick infestations can reduce livestock productivity and cause significant financial losses for farmers. Currently, chemically synthesized acaricides are still the most widely used for controlling ticks. The prolonged and repeated use of these compounds has led to the emergence of tick populations resistant to these substances. Pyridaben, as a mitochondrial electron transport inhibitor, demonstrates excellent efficacy against mites at all developmental stages, though moderate resistance to it has developed with prolonged use (Dimon *et al*., 2026). Imidacloprid selectively targets insect nicotinic acetylcholine receptors, and poses potential toxicity to pollinators and faces challenges related to resistance

evolution and environmental persistence (Buckingham *et al*., 1997).

In view of the limitations of chemical control, environmentally friendly plant-derived acaricides are gaining increasing attention. Plant secondary metabolites such as alkaloids, terpenoids, and flavonoids can target ticks through mechanisms including neurotoxicity, cuticle disruption, and reproductive interference (Rosado *et al*., 2017). Plant secondary metabolites offer advantages such as biodegradability, relatively low impact on non-target organisms, and synergistic delay of resistance through multiple components, thereby supporting sustainable tick control (Wood *et al*., 2026).

Citrus (*C.*) *limon* belongs to the Rutaceae family, and its essential oil (EO) finds extensive applications across multiple industrial sectors, including personal healthcare, perfumery, pharmaceuticals, food, pathogen suppression, and pest control (Agarwal *et al*., 2022; Laudani *et al*., 2022). Research has demonstrated that citral EO exhibits significant acaricidal toxicity against multiple tick species. Citrus EOs exhibit significant acaricidal toxicity against both nymph and adult stages of *H. longicornis* (Gao *et al*., 2022; Nwanade *et al*., 2024). Citrus EOs can reduce the reproductive capacity of *Rhipicephalus* (*R.*) *microplus* female ticks (Pazinato *et al*., 2016). Limonene, as the primary active monoterpene component in *C. limon* EO, has been shown to exhibit significant adulticidal effects against *R. annulatus* and *R. sanguineus*. It also effectively inhibits acetylcholinesterase activity (Prado-Rebolledo *et al*., 2017; Ibrahium *et al*., 2024). *Jasminum* (*J.*) *sambac* is an evergreen shrub belonging to the Oleaceae family and the genus *Jasminum*, renowned for its fragrant flowers. Its constituents are widely utilized in insecticides, cosmetics, and aromatherapy products (Wang *et al*., 2026a). Research on the acaricidal toxicity of *J. sambac* EO remains relatively limited, though its application in agricultural pest control has been documented. *Jasminum sambac* EO exhibits certain toxic and repellent effects against multiple pests such as *Piophila casei* and *Callosobruchus maculatus* (Bi *et al*., 2023; Wanna *et al*., 2023). *Jasminum sambac* EO is rich in terpenoid compounds such as linalool, benzyl acetate, and jasmone, and its composition resembles that of plant essential oils known to possess acaricidal toxicity. Consequently, *J. sambac* EO also holds potential for development as a plant-derived acaricide (Alvares *et al*., 2025; Yang *et al*., 2025).

In this study, the toxic effects of *C. limon* EO, *J. sambac* EO, and their major components against *H. doenitzi* were evaluated using immersion tests, enzyme assays, gene expression analysis, and molecular docking. Based on tick control trials, *C. limon* EO and limonene were selected for further mechanistic investigation. RNA interference (RNAi) was employed to determine whether silencing chitin binding protein (CBP) and fumarylacetoacetase (FAH) genes enhances tick susceptibility to these treatments. In particular, CBP plays an important role in insect growth and defense against environmental stressors (Li *et al*., 2026). Similarly, FAH is also essential: knockdown of this gene results in severe morphological abnormalities and rapid death in the offspring, indicating that FAH is a key gene for maintaining the integrity of insect embryonic development and offspring viability (Sterkel and Oliveira, 2017). Given the need to reduce synthetic acaricide use, the synergistic potential of *C. limon* EO and limonene in combination with synthetic acaricides was assessed. This study aims to contribute to the understanding of the toxic mechanisms of plant-derived secondary metabolites, and to provide a basis for the future development of plant-based acaricides and sustainable tick management strategies.

MATERIALS AND METHODS

Ticks and chemical reagents: Ticks (*H. doenitzi*) at all developmental stages (eggs, larvae, nymphs, and adults) used in this study were obtained from the colony maintained at Hebei Normal University (approval no. LLSC2025008). A total of 50 New Zealand White rabbits (2.5–3.0kg) were used in the experiment; 30 for routine tick rearing, and 20 were used to observe blood-feeding by ticks after immersion treatment (4 rabbits per group). All ticks were cultured under controlled temperature and humidity conditions (26°C, 80% RH). *Jasminum sambac* EO (Commercially available products) and *C. limon* EO were both purchased from Jianhua Natural Essential Oil Mall (https://jian016646.11467.com/). Both are EOs extracted through steam distillation. α-hexylcinnamaldehyde (Cat. No. H811413) and limonene (Cat. No. R902446) were both purchased from McLean Biochemical (Shanghai, China). Imidacloprid and pyridaben were purchased from Hebei Runda, China and Shandong Aodili, China, respectively. Limonene and α-hexylcinnamaldehyde were obtained from Macklin Biochemical, Shanghai, China.

Gas chromatography-mass spectrometry (GC/MS): The chemical composition of *J. sambac* and *C. limon* EOs was analyzed using an Agilent 7890A/5975C gas chromatography-mass spectrometry system (Agilent Technologies, USA). Chromatographic separation was performed using a DB-5MS capillary column (30m×0.25mm×0.25μm). High-purity helium served as the carrier gas at a constant flow rate of 1mL/min. The split ratio was set to 30:1, and the injector port temperature was maintained at 270°C. The column oven employed a programmed temperature ramp: initial temperature of 60°C held for 3min followed by a linear temperature increase of 4°C/min to 220°C, held for 10min. Mass spectrometry detection conditions were as follows: electron impact ionization source energy of 70eV, ion source temperature of 230°C, and mass scan range set to 20–400m/z. Finally, the relative content of each chemical component was calculated using area normalization.

Nymph and adult immersion test: The acaricidal toxicity of the sample was determined using an in vitro immersion method. For the bioassays, groups of 10 unfed nymphs or 10 unfed adults were separately transferred into 1.5mL centrifuge tubes. The test concentration gradients were set as follows: the treatment concentrations of *J. sambac* EO for adults and nymphs were 200, 100, 50, 25, 12.5, and 6.25mg/mL, respectively; the treatment concentrations of *C. limon* EO, limonene, and α-

hexylcinnamaldehyde were all 100, 50, 25, 12.5, 6.25, and 3.125mg/mL. Add 1mL of a series of working solutions prepared with 2% Tween 80 (containing EOs, limonene, and α-hexylcinnamaldehyde) and immerse for 5min. After treatment, transfer ticks to filter paper to dry, then incubate in fresh centrifuge tubes at constant temperature for 24h. Record mortality (criterion: limbs curled and unresponsive to touch). Test solutions were prepared in 2% Tween 80, with the solvent alone as control. Each concentration was applied to 10 ticks per replicate (three replicates per treatment), using a total of 840 nymphs and 840 adults.

Enzymatic assays: To investigate the toxic effects of two EOs, limonene and α-hexylcinnamaldehyde, on the tick *H. doenitzi*, changes in the activity of four key enzymes (AChE, CarE, ATPase, and GST) were measured. Unfed adult ticks were randomly divided into several groups of 50 individuals each, with three replicates per group. Including the control group, a total of 750 ticks were used in the experiment. Each group was immersed in a sublethal concentration of the respective test agent (dissolved in 2% Tween 80) for 5min. The control group was treated with an equal volume of 2% Tween 80 solution. After immersion, the ticks were removed, blotted dry with filter paper, and placed in an incubator for 24h. Subsequently, surviving whole ticks (entire body homogenate) were collected, immediately flash-frozen in liquid nitrogen, and stored at −80°C until further use. The enzyme solution preparation procedure is as follows: Take 25mg of tick tissue, add 250μL of pre-chilled PBS (pH 7.4, containing 1mM PMSF), and homogenize in a tissue grinder (–4°C, 30Hz, 180s). Then centrifuge at 4°C and 10,000×g for 10min, and collect the supernatant as the enzyme solution. All enzyme assays were conducted according to the kit instructions, with three replicates per group. A microplate reader was used for detection, and enzyme activity was calculated based on substrate consumption or product formation per unit time (Zhang *et al*. 2025b).

Gene expression analysis: The experimental and control groups were treated in the same manner as in the enzyme assay. Unfed adult ticks were randomly allocated into groups of 50 ticks each, with three replicates per group (750 ticks total including controls). The effect of the action of limonene and α-hexylcinnamaldehyde on *H. doenitzi* was elucidated using real-time quantitative (qPCR). The qPCR analysis was performed using primers for the GST (*HD-GSTa*), ABC transporter (*HDABCE1*), and CYP450 (*HD-CYP450a*) genes based on previously reported sequences (Zhang *et al*. 2025c). β-actin was used as the internal control gene, and amplification was carried out using TranScript® Green qPCR SuperMix. Primer amplification efficiencies ranged from 90% to 100%, with single melting curve peaks confirming no non-specific amplification or primer-dimer formation. All PCR efficiencies and R^2values were ≥0.98. Each treatment included three biological replicates (10 ticks each), and each sample was tested in triplicate. The amplified products were verified for specificity using 1.5% agarose gel electrophoresis, and the relative expression levels of each gene were calculated based on the $2^{-\Delta\Delta Ct}$ method.

Molecular docking: In the molecular docking section, homology modeling of GST (PQ657472), ABC transporter (XOD50192), and CYP450 proteins (XCD23281) was performed using SWISS-MODEL. The 3D structures of the ligand molecules (limonene and α-hexylcinnamaldehyde) were obtained from the PubChem database. Molecular docking was performed using AutoDockTools 1.5.7 with a grid box (60×60×60points, 0.375Å spacing) centered on the active site, employing the Lamarckian genetic algorithm (150population size, 2.5×10^6evaluations) for 100runs, and the best pose was selected by lowest binding energy and cluster analysis, with interactions visualized in PyMOL.

Assess tick control: To assess the sublethal effects of the test drug on female adult ticks of the species *H. doenitzi*, this study determined its median lethal concentration (LC_{50}) and further analyzed its impact on tick reproductive parameters. Immerse unfed adult female ticks in a solution at the LC_{50} concentration for 3min, then remove them and continue culturing under standard conditions for 24h. The surviving individuals were transferred to a new container after being dried on filter paper, and New Zealand rabbits were provided as hosts for them to feed on. Record the adsorption rate and engorged weight ticks after feeding. Place each engorged female tick individually into an ovipositor and cultivate under constant temperature and humidity conditions. After spawning concludes, the total number of egg masses is recorded. The eggs are collected and cultivated until hatching, at which point the hatch rate is calculated. The solvent (2% Tween 80 aqueous solution) served as the negative control. Each group contained about 50 female and 50 male ticks with three replicates, for a total of approximately 750 females and 750 males. Data were compared with the control group, and appropriate statistical methods were used to analyze significant differences.

Assessing the role of genes in acaricide-stressed ticks via RNAi: To evaluate the effects of *C. limon* EO and limonene on the detoxification and metabolic mechanisms of ticks, this study involved a 3min immersion at the LC_{50} concentration, followed by the detection of expression levels of five relevant genes 24h later. Primers designed based on transcriptomic data (SUB16061975) for use in qPCR, RNAi, and gene cloning (Table S1, Fig. S1). Using tick cDNA as a template, the target RNA fragment was amplified via PCR and subsequently purified using gel extraction. Double-stranded RNA (dsRNA) was synthesized using the T7 RiboMAX *In Vitro* Transcription Kit (Promega, China), with GFP dsRNA (dsGFP) serving as a negative control. Microinjection was performed using a microinjector to inject approximately 0.5μL of dsRNA (5μg/μL) into the ventral side of the ticks. The control group received an injection of an equal volume of GFP dsRNA. At 24h post-injection, total RNA was extracted from the ticks, and the expression levels of the target gene were detected via qPCR to confirm the silencing efficiency. Ticks that survived the disturbance were exposed to LC_{50} limonene and *C. limon* EO for 3min. The 24h mortality rate was recorded to assess the functional role of the target gene under acaricidal stress. At least 540 ticks (60 ticks per biological replicate, three replicates per group) were used for qPCR and RNAi analyses.

Synergism of *C. limon* EO and limonene with acaricides: Ticks were immersed for 5min using the immersion method, and the LC_{50} values of imidacloprid and pyridaben for adult ticks were determined 24h later. Prepare mixtures with volume ratios of 3:1, 1:1, and 1:3. Test each mixture using the LC_{50} values of *C. limon* EO, limonene, and an acaricide by immersing ticks in the solution for 3min and then leaving them for 24h. Three replicates of 10 ticks each were conducted per treatment, totaling 960 ticks. Record the observed mortality rate of the ticks, calculate the expected mortality rate, and the toxicity ratio; a toxicity ratio greater than 1.5 indicates a possible synergistic effect. Based on this, ticks were treated using an in vitro immersion method for 5min, and the mortality rate was recorded 24h later. Each treatment consisted of three replicates with 10 ticks per replicate, accounting for a total of 720 ticks used in the experiment. The potency regression curve and LC_{50} values were calculated, and the co-toxicity coefficient (CTC) was determined. If CTC>120, this indicates a synergistic effect; if 100≤CTC≤ 120, this indicates an additive effect; and if CTC<100, this indicates an antagonistic effect. The formulas for the toxicity ratio and the synergistic coefficient are as follows: Expected mortality rate=(measured mortality rate of EO at its LC_{50} concentration×proportion of EO in the mixture)+(measured mortality rate of the acaricide at its LC_{50} concentration×proportion of the acaricide in the mixture). Toxicity ratio=actual mortality rate/expected mortality rate. Toxicity Index (TI)=(LC_{50} reference substance/LC_{50} of test substance)×100. Actual Toxicity Index (ATI) of the mixture=(LC_{50} substance A/LC_{50} the mixture)×100. Theoretical Toxicity Index (TTI) of the mixture=Agent A's TI×Agent A's concentration in the mixture (%)+Agent B's TI×Agent B's concentration in the mixture (%). Synergistic Coefficient (CTC) = (ATI/TTI)×100.

Statistical analysis: Most statistical analyses were performed using SPSS version 24.0. All data were first subjected to the Shapiro-Wilk normality test and Levene's test for homogeneity of variances. When these conditions were met, one-way ANOVA was used to compare multiple groups, supplemented by Tukey's test for genuinely significant differences (HSD) for post hoc multiple comparisons; Student's t-test was used for comparisons between two groups. LC_{50} values were calculated using Probit regression analysis in the Polopuls software, and the overlap of 95% confidence intervals was used as the criterion for determining statistical significance. A p-value of <0.05 was considered statistically significant. Data are expressed as mean±standard error (SE) from three independent replicates. Molecular docking simulations were carried out using AutoDock software version 4.2 to predict the binding affinities between the ligands and target proteins.

RESULTS

Chemical composition analysis: Significant compositional differences were observed in the chemical analysis of two EOs (Table 1). The *J. sambac* EO exhibits a complex chemical profile, with 13 compounds identified, encompassing structural types such as terpenes, aromatic esters, alcohols, aldehydes, and lactones. Its aroma exhibits rich complexity, primarily composed of key components such as α-hexylcinnamaldehyde (21.71%), limonene (16.07%), benzyl acetate (10.40%), neral (9.73%), and methyl anthranilate (3.17%), forming a typical composite floral bouquet. In contrast, *C. limon* EO exhibits a highly concentrated composition, with 8 compounds identified. Limonene constitutes a dominant 86.76%, complemented by citral (containing 5.01% neral and 5.9% citral), presenting a singular, distinct citrus aroma dominated by limonene.

Nymph and adult immersion test: The immersion test results indicate that the test samples exhibit significant sensitivity differences between nymphs and adults, with all samples demonstrating markedly higher toxicity to nymphs than to adults (Fig. 1). The LC_{50} values for EOs were generally low, and the contact toxicity of their two main monomeric components, α-hexylcinnamaldehyde and limonene, was significantly higher than that of the EO from which they were derived (Table 2). For nymph ticks, the LC_{50} of α-hexylcinnamaldehyde (3.133mg/mL) was significantly lower than that of *J. sambac* EO (31.094mg/mL), while the LC_{50} of limonene (2.784mg/mL) was also markedly lower than that of *C. limon* EO (24.205mg/mL). A similar trend was observed for adult ticks. *Jasmine sambac* EO, α-hexylcinnamaldehyde, *C. limon* EO, and limonene exhibited LC_{50} values of 52.089, 14.206, 23.998, and 27.357mg/mL, respectively. However, no significant difference in toxicity was observed between *C. limon* EO and limonene against adult ticks. Among the tested agents, nymphs were most sensitive to α-hexylcinnamaldehyde, followed by limonene, *C. limon* EO, and *J. sambac* EO. For adult ticks, the order of toxicity was as follows: α-hexylcinnamaldehyde was the most toxic, followed by *C. limon* EO and limonene (with comparable toxicity), while *J. sambac* EO exhibited the lowest toxicity.

Table 1: Chemical composition of *J. sambac* EO and *C. limon* EO.

Essential oil (Extract part)	No.	RT (min)	Constituent	RIa	RIb	Area (%)
J. Sambac	1	5.21	Limonene	1046	1046	16.07
(Dry flower)	2	7.11	Phenylethyl Alcohol	1172	1179	4.48
	3	8.45	Benzyl acetate	1144	1146	10.40
	4	9.21	à-Phenylethyl acetate	1165	1165	1.19
	5	10.68	Neral	1245	1240	9.73
	6	11.57	trans-Citral	1250	1250	13.36
	7	13.61	Dipentene diepoxide	1294	1294	0.29
	8	13.78	Methyl anthranilate	1347	1343	3.17
	9	23.24	Amylcinnamaldehyde	1615	1615	0.37
	10	23.39	Methyl dihydrojasmonate	1657	1657	1.70
	11	24.01	cis-3-Hexenyl salicylatecis3	1665	1664	0.31
	12	26.58	à-Hexylcinnamaldehyde	1762	1762	21.71
	13	29.70	Galoxolide	1913	1913	1.64
C. Limon	1	3.53	β-pinene	958	959	0.71
(Dry peel)	2	4.13	3-Carene	958	948	0.4
	3	4.34	Mycene	1014	1009	0.41
	4	5.21	Limonene	1046	1046	86.76
	5	7.70	α-Pinene	988	987	0.43
	6	10.68	Neral	1074	1174	5.01
	7	11.56	Citral	1241	1249	5.9
	8	45.44	l-(+)-Ascorbic acid 2,6-dihexadecanoate	476	547	650.37

Note: RT: retention time. RIa: Retention index calculated by linear interpolation relative to retention times of a standard mixture of n-alkanes (C7–C40) using a HP-5MS column. RIb: Retention index from literature. Area (%): Percentage of the chemical compound.

Enzymatic assays: To evaluate the effects of two EOs and their major monomeric components on key enzyme activities in adult ticks, enzyme activity assays were conducted after 24h treatment at LC_{50} concentrations (Fig. 2). Compared with the control group (62.78U/g), both *C. limon* EO (18.46±6.95U/g) and α-hexylcinnamaldehyde (20.60±3.08U/g) significantly inhibited Na^+/K^+-ATPase activity, with reductions of 44.32U/g (95% CI:7.46–81.18, P=0.0302) and 42.18U/g (95% CI:25.82–58.54, P=0.0034), respectively. None of the test substances significantly affected GST activity (P>0.05). Limonene significantly inhibited AchE activity (267.2U/g) compared with the control (374.0U/g), with a mean difference of 106.8±17.25U/g (95% CI:53.09–160.6, P=0.0002). Compared with the control group (162.9U/g), both *C. limon* EO (101.6U/g) and limonene (103.6U/g) inhibited CarE activity, with mean reductions of 61.27±10.10U/g (95% CI:16.43–139.0, P=0.0786) and 59.36±3.48U/g (95% CI:32.59–86.13, P=0.0109), respectively.

Table 2: Toxicity of essential oils from *J. sambac* and *C. limon* EO against *H. doenitzi* nymph and adults.

Stage	Treatment	n	df	Regression eq. (Y=)	LC_{30} (95% CI)	LC_{50} (95% CI)	χ^2	Slope±SE
Unfed nymphs	*J. sambac*	180	5	0.3278+0.0074x	17.008[a] (11.570–22.472)	31.094[a] (23.657–40.260)	86.405	2.001±0.271
	α-hexylcinnamaldehyde	180	5	0.0038x+0.7158	1.761[b] (0.580–2.928)	3.133[b] (1.483–4.591)	63.836	2.095±0.440
	C. limon	180	5	0.0177x+0.15	13.365[a] (9.634–17.306)	24.205[a] (18.763–31.772)	90.437	2.033±0.270
	Limonene	180	5	0.0038x+0.7158	0.775[b] (0.041– 2.239)	2.784[b] (0.480–5.634)	15.919	0.944±0.237
Unfed adults	*J. sambac*	180	5	0.0847x+0.0102	34.814[a] (27.346–42.501)	52.089[a] (42.673–64.253)	121.730	2.997±0.378
	α-hexylcinnamaldehyde	180	5	0.0102x+0.2048	10.432[b] (7.497–13.309)	14.206[c] (10.991–18.581)	136.671	3.910±0.524
	C. limon	180	5	0.0889+0.0212x	16.955[b] (12.910–21.107)	23.998[b] (19.203–30.432)	139.637	3.475±0.442
	Limonene	180	5	0.0093x+0.096	15.034[b] (10.553–19.924)	27.357[b] (20.667–37.420)	80.556	2.017±0.267

Note: LC=lethal concentration (mg/mL); CI=confidence interval; df=degree of freedom. In each stage the data followed with different letter of each column are significantly different at P<0.05; χ^2 =Chi-square value. For each stage, different letters indicate significant differences among treatments (P<0.05).

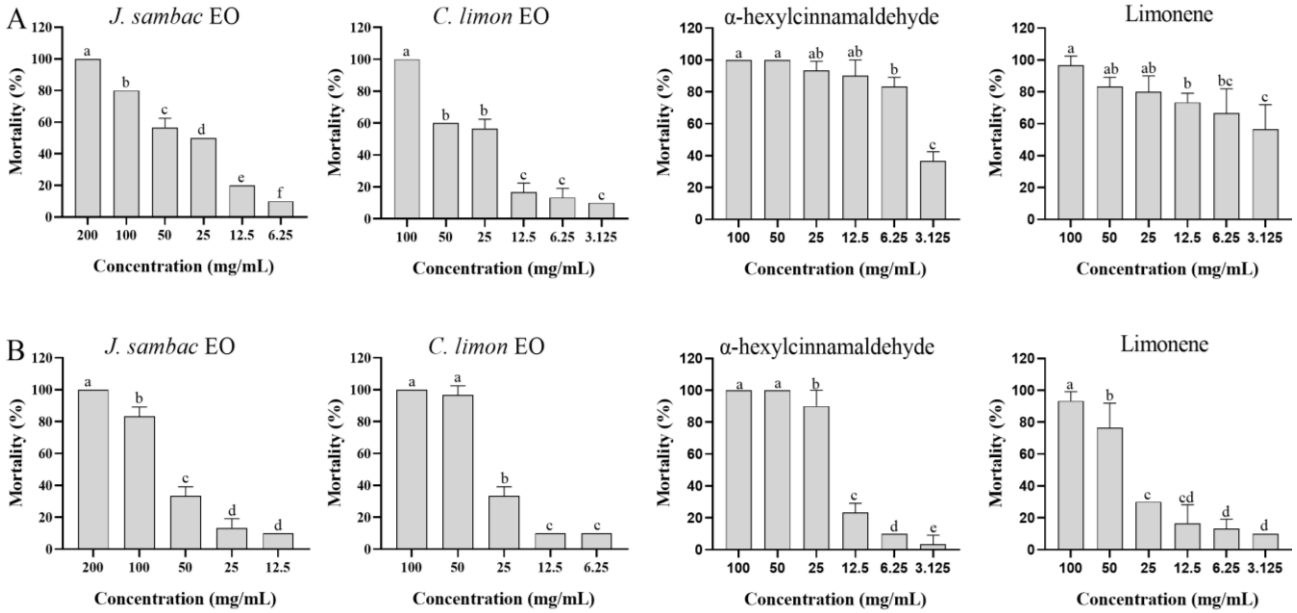


Fig. 1: Contact toxicity of two EOs, limonene and α-hexylcinnamaldehyde against *H. doenitzi* unfed ticks. A: Nymph tick; B: Adult tick (5min exposure, 24h post-treatment). Different letters indicate statistically significant differences (P<0.05). Data are mean ± SE from three biological replicates (n=3).

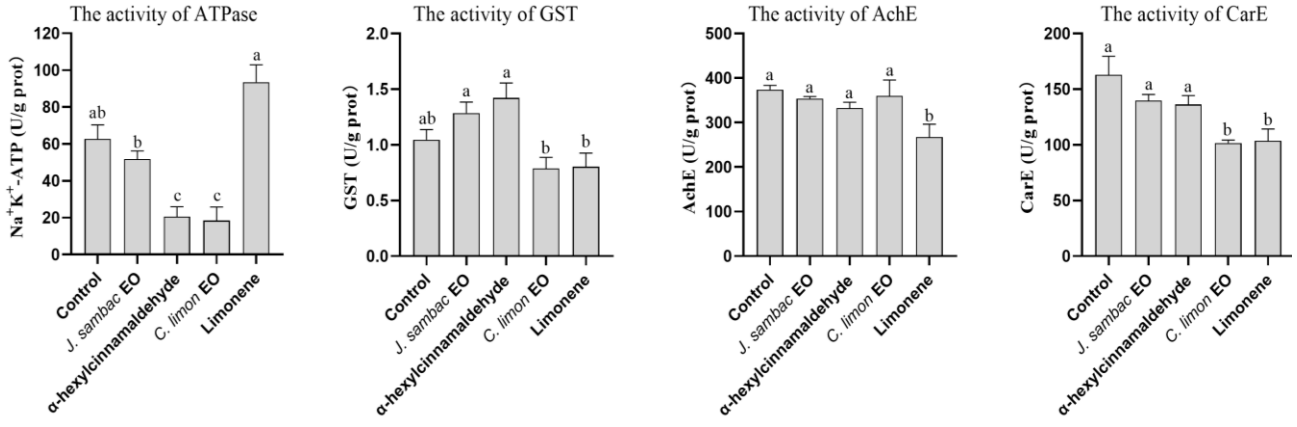


Fig. 2: Effects of two EOs, limonene and α-hexylcinnamaldehyde on the activities of ATPase (Na^+/K^+-ATPase), GST (Glutathione S-transferase), AchE (Acetylcholinesterase), and CarE (Carboxylesterase) in *H. doenitzi* adults. Enzyme activities were measured 24h after immersion treatment (5min exposure). Different letters indicate statistically significant differences (P<0.05). Data are mean±SE from four biological replicates (n=4).

Gene expression analysis: For assessing the effects of EOs and major monomers on the expression of tick detoxification enzyme genes, this study measured the expression levels of three relevant genes 24h after treatment at the LC_{50} concentration (Fig. 3). Compared with the control group, treatment with *C. limon* EO and limonene significantly upregulated the expression of ABC transporter gene, by 5.624-fold (P=0.0045) and 34.48-fold (P=0.0039), respectively. The GST gene exhibited high expression in all treatment groups. Compared with the control group, the mRNA expression levels of genes in response to *J. sambac* EO, α-hexylcinnamaldehyde, *C. limon* EO, and limonene were upregulated by 3.26-fold (P=0.0014), 8.60-fold (P<0.0001), 1.53-fold (P=0.0052), and 19.28-fold (P=0.0004), respectively. The expression of the CYP450 gene was upregulated 4.71-fold (P=0.0004) by limonene treatment, whereas it was downregulated by −0.406-fold (P=0.0230), −0.468-fold (P=0.0002), and −0.702-fold (P=0.0011) following treatments with *J. sambac* EO, α-hexylcinnamaldehyde, and *C. limon* EO, respectively. This may be related to antagonistic interactions among the complex components of EOs.

Molecular docking: Molecular docking results indicate (Fig. 4) that the binding energies of α-hexylcinnamaldehyde with ABC transporter, CYP450, and GST are −2.71, −2.14, and −2.56kcal/mol, respectively; while limonene exhibited binding energies of −4.20, −3.79, and −3.77kcal/mol for the three enzymes, demonstrating the strongest binding affinity for ABC transporter. Visual analysis reveals that α-hexylcinnamaldehyde achieves stable binding with each target protein by forming hydrogen bonds with GLU-341 and MET-552 of ABC transporter, THR-94 and LYS-146 of GST, and ARG-87 of CYP450.

Assess tick control: To investigate the effects of EOs and their major components on tick reproductive development, we measured key parameters including feeding time, engorged weight, egg mass weight, and hatching rate (Fig. 5). Compared with the control group, both *J. sambac* EO and limonene significantly prolonged tick feeding duration by 0.7375d (P<0.0001) and 0.4918d (P=0.0270), respectively. In terms of engorged weight, *J. sambac* EO significantly increased tick weight by 45.81mg (P<0.0001), whereas *C. limon* EO, α-hexylcinnamaldehyde, and limonene decreased it by 57.20mg (P<0.0001), 39.29mg (P<0.0001), and 11.98mg (P=0.4754), respectively. Compared with the control group (102.1mg), *C. limon* EO significantly reduced egg weight to 70.97mg (mean difference=31.11mg, P=0.0005), whereas α-hexylcinnamaldehyde (81.50mg) also significantly decreased egg weight (mean difference=0.58 mg, P=0.0010). However, limonene did not show a significant effect (92.69mg, mean difference=9.393 mg, P=0.4358). Overall, *C. limon* EO and limonene exhibited inhibitory effects on tick reproduction and development, while *J. sambac* EO demonstrated a certain degree of promotion. Based on these findings, subsequent studies conducted multi-omics integrated analyses on *C. limon* EO and limonene to systematically elucidate their mechanisms of action.

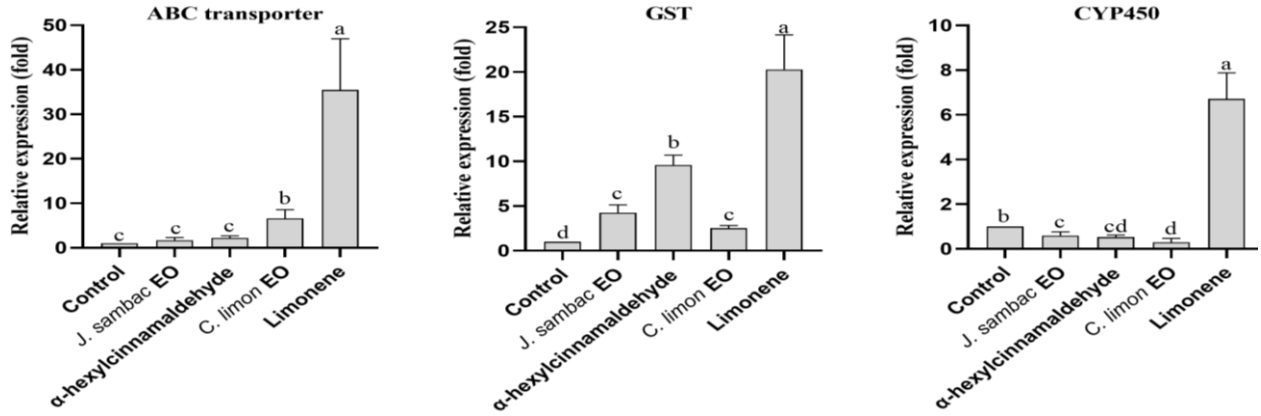


Fig. 3: Relative mRNA expression levels of ABC transporter (*HDABCE1*), GST (*HD-GSTa*), and CYP450 (*HD-CYP450a*) genes in *H. doenitzi* adults following treatment with two EOs, limonene and α-hexylcinnamaldehyde. Different letters indicate statistically significant differences (P<0.05). Data are mean±SE from six biological replicates (n=6).

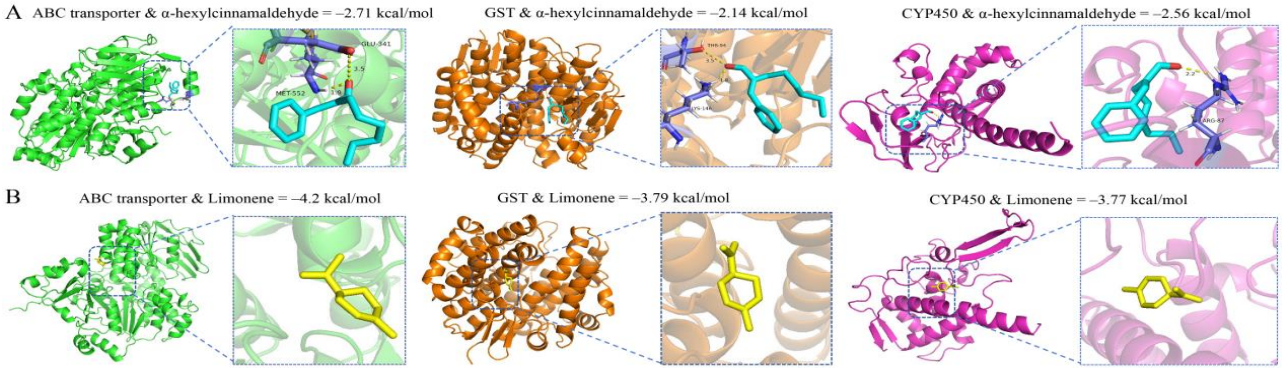


Fig. 4: Schematic representation of the interaction of compounds with *H. doenitzi* ABC transporter, GST, and CYP450. A: α-hexylcinnamaldehyde; B: Limonene. Hydrogen bonds and hydrophobic interactions are shown as dashed lines and surface representations, respectively. Docking was performed using AutoDock 4.2.

Assessing the role of genes in acaricide-stressed ticks via RNAi: To identify key genes involved in the tick's response to *C. limon* EO and limonene stress, the expression levels of five candidate genes were validated using qPCR (Fig. 6A). The expression levels of chitin binding protein (CBP) genes were significantly upregulated by 1.70-fold and 0.98-fold in *C. limon* EO and limonene treatment groups, respectively (P<0.0001). The fumarylacetoacetase (FAH) gene was downregulated by 0.54-fold and 0.90-fold under the two treatment conditions, respectively (P<0001). The heat shock 70kDa protein (HSP70) gene was downregulated by 0.50-fold and 0.26-fold, respectively (P<0007). The UDP-4-amino-4-deoxy-L-arabinose aminotransferase (UDP-AT) genes were downregulated by 0.75-fold and 0.48-fold, respectively (P<0001). The cyclophilin ABH-like (CYC) genes were significantly upregulated by 2.5-fold and 1.0-fold in the *C. limon* EO and limonene treatment groups, respectively (P<0001).

Based on the aforementioned quantitative expression profiles and their potential functional correlations, CBP and FAH were selected for functional validation. First, these two genes were successfully cloned (Fig. S1) and named HDCBPa (PZ311692) and HDFAHa (PZ311693), respectively. Sequence analysis revealed that HDCBPa encodes 131 amino acids, while HDFAHa encodes 419 amino acids (Fig. 7A). Structure prediction indicates that HDCBPa consists primarily of α-helices and random coils, with several β-sheets distributed in its C-terminal region. In contrast, HDFAHa exhibits a typical α/β-hydrolase fold, characterized by a central β-sheet surrounded by α-helices (Fig. 7B). Multiple sequence alignment analysis revealed that HDCBPa shares 100% similarity with the corresponding sequence from *H. longicornis*, while HDFAHa exhibits a lower similarity of approximately 66% with protein sequences from moths, suggesting a close genetic relationship between the two. Phylogenetic analysis further confirmed that the protein from the tick formed an independent clade, clearly distinct from proteins of other species (Fig. 7C).

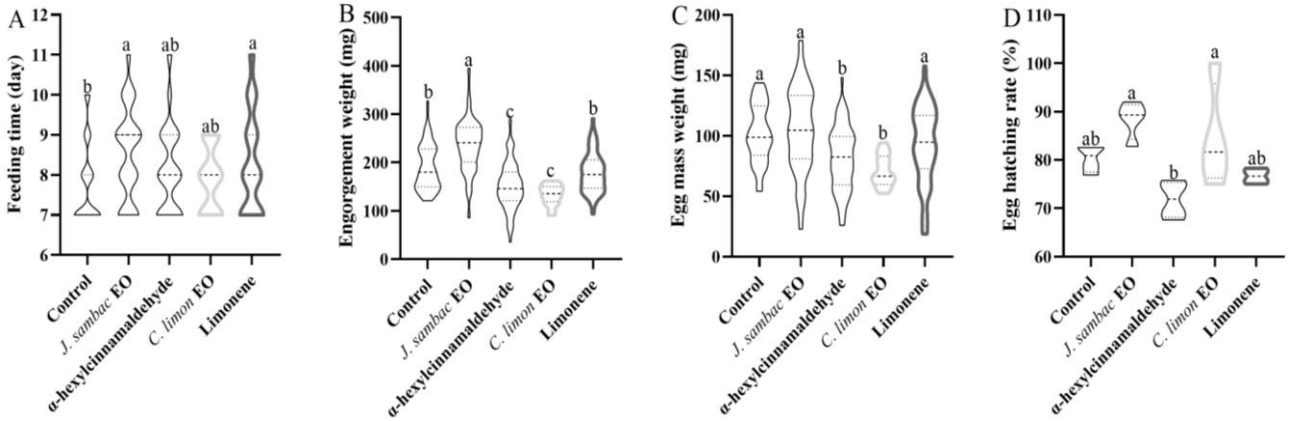


Fig. 5: The effects of a contact treatment involving two EOs, limonene and α-hexylcinnamaldehyde, on the biological characteristics of *H. doenitzi*. A: Feeding time; B: Engorgement weight; C: Egg mass weight; D: Egg hatching rate. Different letters indicate statistically significant differences (P<0.05). Data are mean±SE from six biological replicates (n=6).

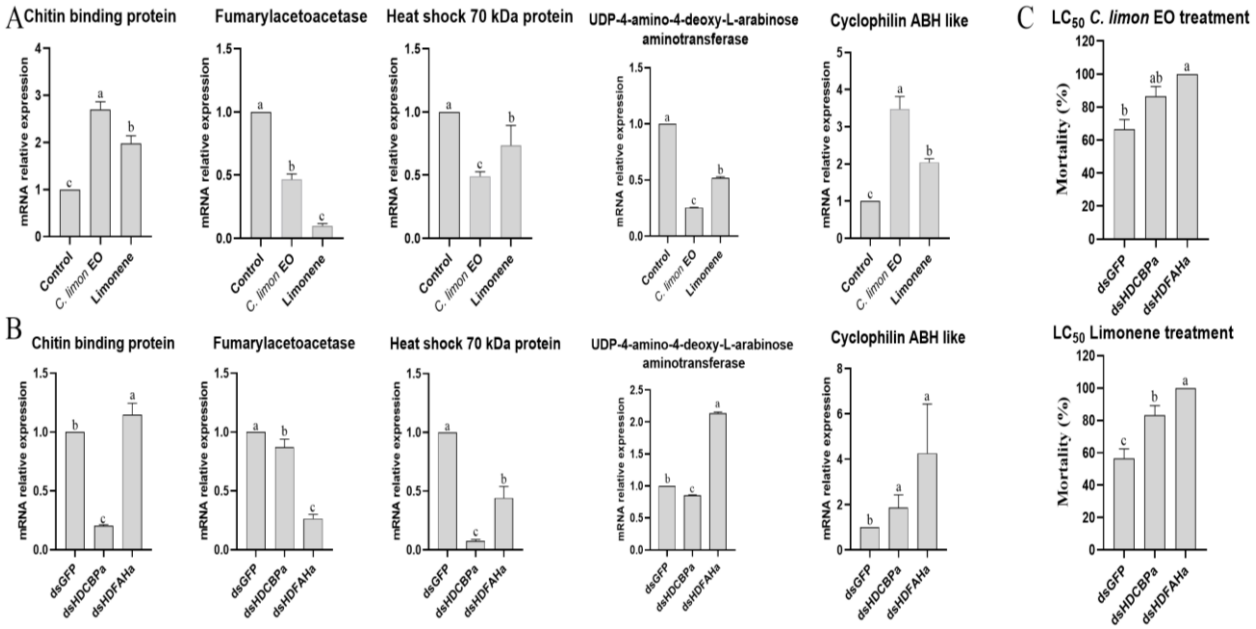


Fig. 6: A: Relative gene expression levels in unfed adult *H. doenitzi* treated with *C. limon* EO and limonene. B: Relative expression of target genes in unfed adult *H. doenitzi* after dsRNA-mediated knockdown of *HDCBPa* and *HDFAHa* (GFP, green fluorescent protein). C: Tick mortality after *C. limon*

EO and limonene treatment following RNAi-mediated gene knockdown. Different letters indicate statistically significant differences (P<0.05). Data are mean±SE from six biological replicates (n=6).

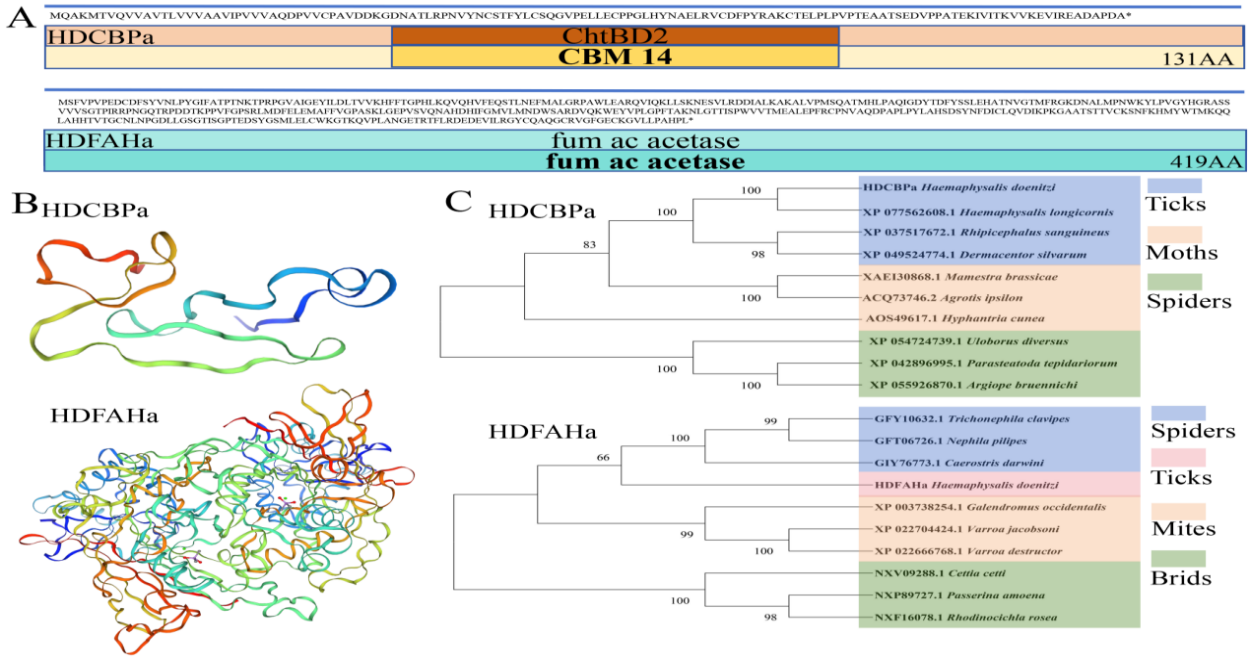


Fig. 7: Bioinformatic analysis of two proteins characterized from *H. doenitzi*. A: Schematic diagram of the conserved structure domains of proteins. B: The predicted tertiary structures; C: Phylogenetic tree of the amino acid sequences. Phylogenetic tree constructed using the neighbor-joining method (1,000 bootstrap replicates) based on amino acid sequences.

To validate the functions of the *HDCBPa* and *HDFAHa* genes, dsRNA targeting these genes was synthesized and injected into adult ticks. 24h after injection, gene silencing efficiency was assessed by qPCR (Fig. 6B). Compared with the control group injected with dsGFP, the transcription level of the target gene was reduced by 79.7% in the dsHDCBPa group (P<0.0001); in the dsHDFAHa group, the transcription level of the target gene was reduced by 73.6% (P<0.0001). Following *HDCBPa* knockdown, the expression levels of *HDFAHa*, HSP70, and UDP-AT all decreased, while the expression level of the CYC gene increased. Following *HDFAHa* knockdown, HSP70 gene expression decreased, while the expression levels of *HDCBPa*, UDP-AT, and CYC all increased. To determine whether *HDCBPa* and *HDFAHa* exert a protective effect against *C. limon* EO and limonene exposure, gene-silenced ticks were exposed to LC_{50} *C. limon* EO or limonene for 5min, and mortality rates were recorded 24h later (Fig. 6C). In the dsHDCBPa group, the mortality rate was 86.7% after treatment with *C. limon* EO and 83.3% after treatment with limonene, both significantly higher than that of the control group. In the dsHDFAHa group, the mortality rate reached 100% after treatment with either *C. limon* EO or limonene, significantly higher than the 45.2% observed in the control group *(*P<0.05). These results indicate that knocking down the *HDCBPa* or *HDFAHa* genes significantly increases the sensitivity of ticks to *C. limon* EO and limonene stress, suggesting that these two genes play an important protective role in helping ticks resist stress from plant-derived acaricides.

Synergism of *C. limon* EO and limonene with acaricides: The toxicity of imidacloprid and pyridaben against adult ticks was determined (Fig. 8A). The LC_{50} value for imidacloprid is 0.903mg/mL, and that for pyridaben is 5.856mg/mL (Table 3). Among these, imidacloprid was significantly more toxic than pyridaben. Preliminary tests determined that the LC_{50} of *C. limon* EO and limonene for adult ticks were 23.998 and 27.357mg/mL, respectively. LC_{50} *C. limon* EO and LC_{50} limonene were mixed with LC_{50} imidacloprid and LC_{50} pyridaben in volume ratios of 1:3, 1:1, and 3:1, respectively. The mixtures were applied for 3min, and mortality rates were recorded 24h later (Fig. 8B). The results showed that when LC_{50} *C. limon* EO and LC_{50} imidacloprid were mixed in a 1:3 ratio, the mortality rate was 93%, which was significantly higher than that of LC_{50} imidacloprid group (40%) and LC_{50} *C. limon* EO group (56.67%) (P<0.05). The mortality rate in the group treated with a 1:3 mixture of LC_{50} *C. limon* EO and LC_{50} pyridaben was 93%, significantly higher than that in the LC_{50} pyridaben group (44.33%) and the LC_{50} *C. limon* EO group (56.67%) (P<0.05). The mortality rate in the 1:3 mixture of LC_{50} *C. limon* EO and LC_{50} pyridaben reached 100%, which was significantly higher than that in the LC_{50} pyridaben group (44.33%) and the LC_{50} limonene group (56.67%) (P<0.05). The expected mortality rates and toxicity ratios were calculated (Table S2). The toxicity ratio was 2.04 (>1.5), when LC_{50} *C. limon* EO was mixed with LC_{50} imidacloprid in a 3:1 ratio, indicating significant synergistic effects. When LC_{50} limonene and LC_{50} imidacloprid are mixed in a 3:1 ratio, the toxicity ratio reaches a maximum of 1.46. When LC_{50} *C. limon* EO and LC_{50} pyridaben are mixed in a 1:3 ratio, the toxicity ratio reaches a maximum of 2.02. When LC_{50} limonene and LC_{50} pyridaben are mixed in a 3:1 ratio, the toxicity ratio reaches a maximum of 2.00. The above ratios represent the optimal synergistic combinations. The mortality rates of ticks were determined for each ratio (Fig. S2), and the combined toxicity coefficient (CTC) of the mixture at the optimal ratio was calculated (Table S3). When LC_{50} *C. limon* EO was mixed with LC_{50} pyridaben

in a 1:3 ratio, the CTC value was 129.71 (>120), indicating a synergistic effect; when LC_{50} limonene was mixed with LC_{50} pyridaben in a 1:3 ratio, the CTC value was 116.0 (100–120), indicating an additive effect.

Table 3: Toxicity of pyridaben and imidacloprid against *H. doenitzi* adults.

Stage	Treatment	n	df	Regression eq. (Y=)	LC_{50} (95% CI)	LC_{90} (95% CI)	χ^2	Slope±SE
Unfed adults	Pyridaben	180	5	0.0167x+0.3322	5.856[a] (4.459–7.531)	15.689[a] (11.570–25.541)	110.363	2.994±0.373
	Imidacloprid	180	5	0.1169x+0.2998	0.903[b] (0.589–1.302)	4.866[a] (2.937–12.608)	68.526	1.752±0.253

Note: LC=lethal concentration (mg/mL); CI=confidence interval; df=degree of freedom. In each stage the data followed with different letter of each column are significantly different at P<*0.05*; χ^2 =Chi-square value.

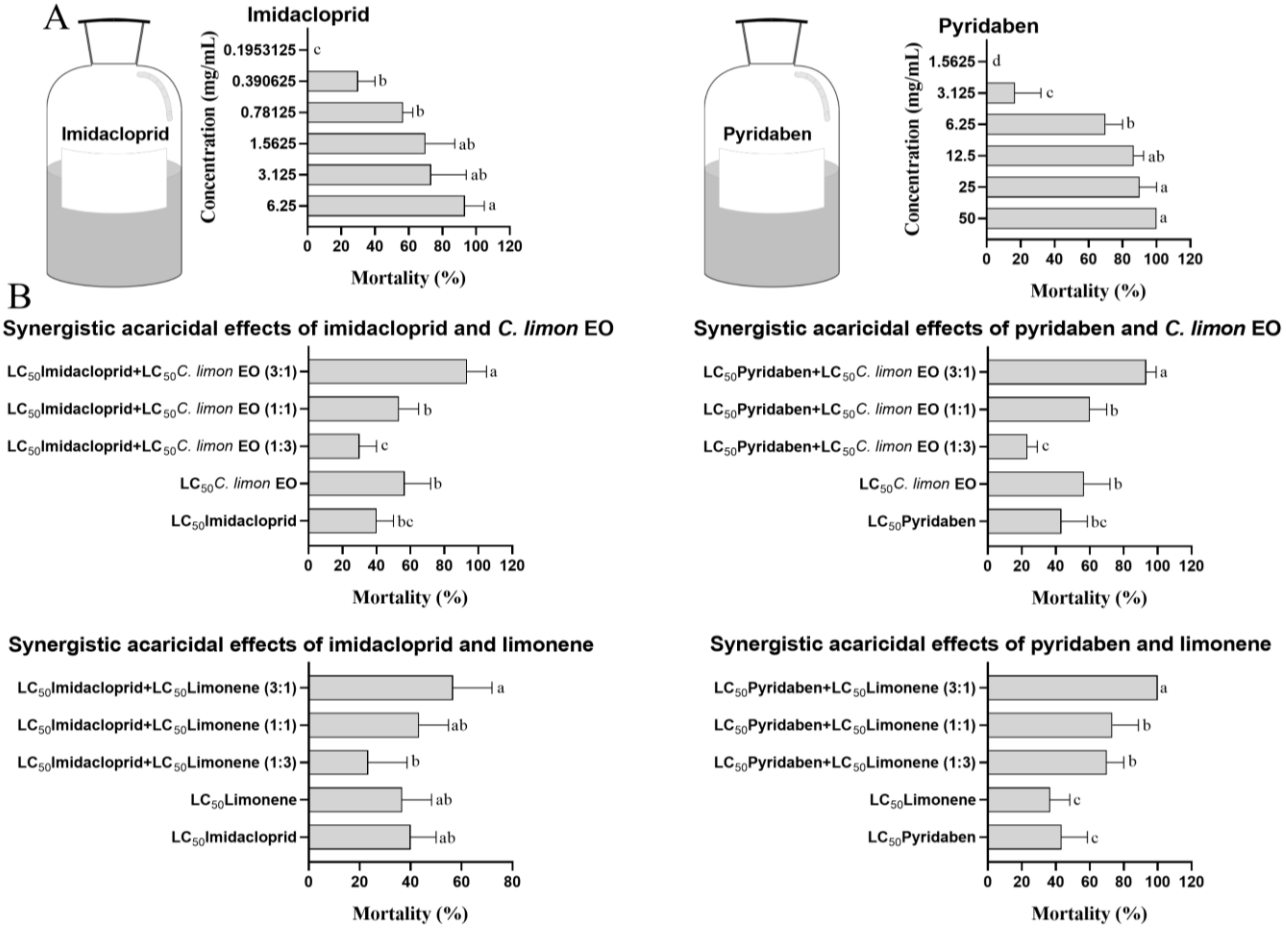


Fig. 8: A: Contact toxicity of imidacloprid and pyridaben against *H. doenitzi* unfed adult ticks (5min exposure, 24h post-treatment). B: Synergistic acaricidal effects of *C. limon* EO and limonene in combination with imidacloprid and pyridaben against *H. doenitzi*. Different letters indicate statistically significant differences (P<0.05). Data are mean ± SE from three biological replicates (n=3).

DISCUSSION

Haemaphysalis doenitzi is an important ectoparasite that poses a threat to human health and the development of the livestock industry, and is widely distributed in regions associated with bird migration (Chitimia-Dobler *et al*., 2024; Zhang *et al*., 2024b). In this study, a commercial *J. sambac* EO, along with *C. limon* EO and their major components, exhibited strong acaricidal toxicity against *H. doenitzi*. Among these, *C. limon* EO has shown superior application potential as it reduces the engorgement weight and egg mass weight of ticks and enhances the efficacy of the chemical acaricide pyridaben.

GC-MS analysis indicated that *C. limon* EO consists primarily of limonene, a finding consistent with the results of numerous analyses of extracted EOs (Petretto *et al*., 2023). In contrast, *J. sambac* EO is dominated by the synthetic compound α-hexylcinnamaldehyde, while also containing natural components such as benzyl acetate and limonene, which aligns with recent analyses of commercial jasmine EOs (Liu, 2022; Fan *et al*., 2025). Immersion tests to determine the LC_{50} values for adult ticks showed that *C. limon* EO (23.998mg/mL) was more toxic than *J. sambac* EO (52.089mg/mL). The LC_{50} value (14.206mg/mL) of α-hexylcinnamaldehyde, the primary component of *J. sambac* EO, is lower than that of limonene (27.357mg/mL). The overall acaricidal toxicity of *J. sambac* EO may be influenced by other trace components or antagonistic interactions among components (Mei *et al*., 2024). Previous studies have shown that *C. limon* EO exhibits significant fumigant toxicity against nymphs and adults of *H. longicornis* (Gao *et al*., 2022). A *C. limon* EO nanoemulsion also demonstrated significant acaricidal toxicity against *R. annulatus* (Ibrahium *et al*., 2022). Furthermore, *C. limon* EO exhibits contact toxicity, fumigant toxicity, and repellent effects against *Musca domestica* larvae (Villanueva-Pereira *et al*., 2025). Limonene nanoemulsions exhibit significant acaricidal toxicity against both the larvae and adults of *R. annulatus* and *R. sanguineus* (Ibrahium *et al*., 2024). However, the

acaricidal activity of *C. limon* EO and limonene against *H. doenitzi*, as well as their underlying mechanisms and synergistic potential with chemical acaricides, remain poorly understood.

Carboxylesterase (CarE), an important detoxifying enzyme in arthropods, protects the organism from harm by metabolizing exogenous toxic substances (Ji *et al*., 2025). Experiments have revealed that both *C. limon* EO and limonene can significantly inhibit CarE activity, which may contribute to the reduction of detoxification capacity in ticks. Additionally, the two EOs and their main components, limonene and α-hexylcinnamaldehyde, significantly upregulated the expression of the glutathione S-transferase (GST) gene. AchE is essential for terminating cholinergic neurotransmission in ticks, and its inhibition would be expected to cause neuromuscular disruption and mortality (Carson *et al*., 1987). The lack of AchE inhibition by the crude EO, in contrast to the significant inhibition observed with limonene alone, suggests that other minor constituents in the oil may antagonize or competitively interfere with the binding of limonene to the AchE active site, thereby masking its inhibitory effect (Zhang *et al*., 2026). As a vital detoxifying and antioxidant enzyme, GST may be upregulated, indicating a detoxification response of ticks under stress (Yuan *et al*., 2026). Both *C. limon* EO and limonene treatments increased the expression of the ABC transporter gene, which is associated with intracellular detoxification and stress adaptation (Pfannenstiel *et al*., 2026). In particular, the fold increase of limonene (34.48-fold) was significantly higher than that of *C. limon* EO (5.62-fold), suggesting that limonene alone may induce a more potent stress response. The upregulation of ABC transporters can enhance tick resistance (Quintana *et al*., 2026). Molecular docking results further support these findings, indicating that limonene binds more strongly to ABC transporters, GSTs, and CYP450 enzymes than α-hexylcinnamaldehyde. CYP450 plays a key, universal detoxification role in resistance to various insecticides, as evidenced by its high expression in the detoxification tissues of *Spodoptera frugiperda* larvae, its upregulation in response to multiple insecticides, and its strong binding affinity to these compounds (Guo *et al*., 2026). These results, supported by enzyme activity and gene expression data, provide a multi-level mechanistic understanding.

A tick control trial revealed a key difference between the two EOs: *J. sambac* EO increased the engorgement weight of ticks, while *C. limon* EO reduced both engorgement weight and egg mass weight. These results indicate that *J. sambac* EO exhibited acaricidal toxicity, while surviving ticks showed increased engorgement weight. In separate studies, *J. sambac* EO has shown potential for development as a behavioral modulator, exhibiting the highest electrophysiological activity against small worker ants of the red imported fire ant (with an EAG value of 1.8788mV) (Li *et al*., 2022a). This phenomenon suggests that *J. sambac* EO may have dual biological effects. On the one hand, it exerts acute toxicity on target arthropods; on the other hand, at sublethal concentrations, certain active components may trigger feeding- or reproduction-related physiological compensatory responses (Calabrese *et al*., 2024; Ullah *et al*., 2025). This hormesis suggests that if surviving ticks consume more blood as a result of compensatory effects, the risk of pathogen transmission could theoretically increase; therefore, careful evaluation is required in practical applications. Similarly, lemon leaf extract reversibly inhibits spermatogenesis and fertility in male mice, a mechanism associated with reduced testosterone levels and the induction of germ cell apoptosis (Singh *et al*., 2016). This study found that *C. limon* EO not only eliminates ticks but also reduces their ability to feed on blood, making it a more attractive candidate for integrated tick management.

The quantitative PCR results showed that, compared with the control group, treatment with *C. limon* EO and limonene resulted in varying degrees of change in the expression levels of multiple functional genes within the ticks. The CBP gene was upregulated 1.7-fold and 0.98-fold following treatment with *C. limon* EO and limonene, respectively. This protein plays a role in maintaining the structure of the tick's cuticle and peritrophic membrane, and its upregulation may represent a stress response to strengthen its physical barrier against chemical stressors (Jin *et al*., 2026). The gene FAH was downregulated by 0.54-fold and 0.90-fold following treatment with *C. limon* EO and limonene, respectively, demonstrating distinct regulatory patterns. FAH is a key enzyme in the tyrosine metabolic pathway, and its differential expression suggests that limonene may induce a stronger stress response by interfering with this pathway (Ren *et al*., 2026). In contrast to the typical pattern in *Aedes aegypti*, where HSP70 is induced under stress to maintain protein homeostasis, the HSP70 gene was downregulated following treatment with both *C. limon* EO and limonene in this study. This may indicate that, under the current experimental conditions, the protein folding stress response in ticks has not been significantly activated, or that the cells have entered a phase of stress adaptation, thereby reducing their dependence on HSP70 (Aciole *et al*., 2026). The UDP-AT gene was downregulated under both treatments, but showed a weak association with the stress response in ticks, suggesting that it may not be a key response gene (Aoyagi *et al*., 2015). The expression levels of the CYC gene increased under both treatment conditions. Given the important role of cyclopeptidases in fungal pathogenicity and host defense, the upregulation of this gene may indicate its involvement in the tick's defense or stress response to plant-derived acaricides (Hwa *et al*., 2011). CBP and FAH are involved in the maintenance of physical barriers and energy metabolism, respectively. Impairments in their function can increase tick susceptibility to acaricides, making them potential targets for tick control via RNAi.

This study further validated the functional importance of *HDCBPa* and *HDFAHa* through RNAi experiments, analogous to the mechanism by which the cuticular protein *PcCPR12* in *Anonychus citri* maintains cuticular integrity by regulating chitin and lipid metabolism (Wang *et al*., 2026b). After silencing these two genes, the ticks exhibited significantly increased sensitivity to *C. limon* EO and limonene, along with significantly elevated mortality, confirming their critical role in maintaining cuticle integrity and defending against chemical stress (Li *et al*., 2022b). This suggests that these

two genes could serve as potential molecular targets for RNAi-based acaricides. Although imidacloprid and pyridaben are frequently detected in pet urine, with some exposure levels exceeding reference safety values, they still demonstrate good acaricidal toxicity against ticks, suggesting that a careful balance must be struck between efficacy and exposure risk (Jongejan *et al*., 2016). This finding is consistent with previous reports that the combination of 10% imidacloprid and 4.5% flumethrin effectively controls tick infestations and pathogen transmission under field conditions. This study found that a 1:3 mixture of *C. limon* EO and pyridaben exhibited a synergistic effect (CTC=129.71), indicating that the combined use of plant-derived compounds and chemical acaricides can also significantly enhance tick control efficacy (Dantas-Torres *et al*., 2013; Labruna *et al*., 2023). Such formulation strategies can reduce the use of chemical acaricides. In summary, *C. limon* EO and limonene showed acaricidal activity, accompanied by inhibition of CarE activity, upregulation of ABC transporter and GST genes, and strong enzyme binding; however, the exact roles and potential mechanisms require further investigation. They reduce tick engorgement weight and egg mass weight while enhancing the efficacy of pyridaben, highlighting their significant potential for sustainable tick control. Future research should focus on field efficacy validation, formulation optimization, and comprehensive safety assessments, including effects on non-target organisms, mammalian toxicity, dermal irritation, and environmental persistence. These efforts are essential to evaluate the practical applicability and ecological safety of *C. limon* EO and limonene as plant-based acaricides before their potential use in tick control programmes.

Conclusions: This study systematically evaluated the acaricidal efficacy and synergistic potential of *C. limon* EO and its major component, limonene, against the tick *H. doenitzi*. The results indicate that both *C. limon* EO and limonene exhibit strong acaricidal efficacy. Specifically, *C. limon* EO reduces the engorgement weight and egg mass weight, whereas *J. sambac* EO has the opposite effect. RNAi studies have confirmed that silencing the *HDCBPa* and *HDFAHa* genes significantly increases the sensitivity of ticks to *C. limon* EO and limonene. Synergy assessments indicate that a 1:3 mixture of *C. limon* EO and pyridaben exhibits strong synergistic effects. In conclusion, *C. limon* EO and limonene show considerable potential for tick control. Further research is needed to verify their feasibility and ecological safety under field conditions.

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Data availability: Data will be made available on request.
Ethics Statement: All animal experiments involving ticks and New Zealand white rabbits were approved by the Animal Ethics Committee of Hebei Normal University (approval no. LLSC2025008).

Authors contribution: S.Z.: Writing – original draft, Writing – review & editing, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition. Z.L.: Validation, Supervision, Resources. Y.L.: Methodology, Investigation. X.Y.: Writing – review & editing, Funding acquisition, Supervision, Software, Project administration, Methodology.

Generative AI statement: The authors declare that generative AI tools were used only for language editing and improvement of readability. The authors take full responsibility for the content of the manuscript.

REFERENCES

Aciole BCTA, Quel NG, Antonio LM, *et al*., 2026. *Aedes aegypti* Hsp70 has enhanced ATPase and protein protection under oxidative stress. International Journal of Biological Macromolecules 352:151091.

Agarwal P, Sebghatollahi Z, Kamal M, *et al*., 2022. Citrus essential oils in aromatherapy: therapeutic effects and mechanisms. Antioxidants (Basel, Switzerland) 11:2374.

Alvares FBV, Ferreira LC, Silva JO, *et al*., 2025. Acaricidal efficacy of the monoterpene linalool against the cattle tick *Rhipicephalus microplus* and its synergistic potential with cypermethrin. Experimental and Applied Acarology 94:45.

Aoyagi KL, Brooks BD, Bearden SW, *et al*., 2015. LPS modification promotes maintenance of *Yersinia pestis* in fleas. Microbiology (Reading, England) 161:628–638.

Bi S, Liu L, Jia M, *et al*., 2023. Exploring insecticidal properties and acetylcholinesterase inhibition by three plant essential oils against the cheese skipper *Piophila casei* (Diptera: Piophilidae). Industrial Crops and Products 203:117198.

Bonizzoni M and Lahondère C, 2026. Blood-sucking arthropods in the Anthropocene: climate change thermotolerance, and global disease risks. Current Opinion in Insect Science 75:101506.

Buckingham S, Lapied B, Corronc H, *et al*., 1997. Imidacloprid actions on insect neuronal acetylcholine receptors. The Journal of Experimental Biology 200:2685-2692.

Calabrese EJ, Nascarella M, Pressman P, *et al*., 2024. Hormesis determines lifespan. Ageing Research Reviews 94:102181.

Carson KA, Sonenshine DS, Boland LM, *et al*., 1987. Ultrastructural localization of acetylcholinesterase in the synganglion of the tick, *Dermacentor variabilis* (Say). Cell and Tissue Research 249:615–623.

Chitimia-Dobler L, Barboutis C, Bounas A, *et al*., 2024. Discovery of a novel mediterranean *Haemaphysalis* (*Ornithophysalis*) *doenitzi* group tick species infesting *Falco eleonorae* on Antikythira Island, Greece. Parasitology 151:933–945.

Dantas-Torres F, Capelli G, Giannelli A, *et al*., 2013. Efficacy of an imidacloprid/flumethrin collar against fleas, ticks and tick-borne pathogens in dogs. Parasites & Vectors 6:245.

Dimon WEOG, Carrillo D, Da CM, *et al*., 2026. Adjuvant-enhanced acaricidal solutions to overcome foliar hydrophobicity for controlling the litchi erinose mite (*Aceria litchii*). Experimental and Applied Acarology 96:22.

Fan W, Liao Z, Gu M, *et al*., 2025. Pan-genome of *Jasminum sambac* reveals the genetic diversity of different petal morphology and aroma-related genes. Molecular Ecology Resources 25:e70013.

Gao Z, Yu Z, Qiao Y, *et al*., 2022. Chemical profiles and enzyme-targeting acaricidal properties of essential oils from *Syzygium aromaticum*, *Ilex chinensis* and *Citrus limon* against *Haemaphysalis longicornis* (Acari: Ixodidae). Industrial Crops and Products 188:1–10.

Guo Z, Tang J, Ling S, *et al*., 2026. Upregulation of a cytochrome P450 gene, *CYP6B50*, confers multi-insecticide resistance in *Spodoptera frugiperda*. Journal of Advanced Research 82:99–111.

Husin NA, Abdulrahim MHS, Halim MRA, *et al*., 2026. Tick-host associations across contrasting habitats in tropical Peninsular Malaysia. Parasites & Vectors 19:99.

Hwa LF, Fakhrana IN, Shaharuddin NA, *et al*., 2011. A partial-length cyclophilin-encoding (CYP) cDNA isolated from ganoderma boninense. Journal of Oil Palm Research 23:1115–1120.

Ibrahium SM, Abdel-Baki AS, Al-Quraishy S, *et al*., 2024. Efficacy of d-limonene nanoemulsion against *Rhipicephalus annulatus* and *Rhipicephalus sanguineus* ticks. Acta Parasitologica 69:267–274.

Ibrahium SM, Wahba AA, Farghali AA, *et al*., 2022. Acaricidal activity of tea tree and lemon oil nanoemulsions against *Rhipicephalus annulatus*. Pathogens (Basel, Switzerland) 11:1506.

Ji Y, Yan J, Palli SR, *et al*., 2025. Identification of carboxylesterase genes and function of three inducible α-esterase genes involved in insecticidal activity of α-solanine in *Phthorimaea operculella*. Journal of Agricultural and Food Chemistry 73:19764–19777.

Jin L, Rong P, Xu M, *et al*., 2026. HaGATAe regulates *chitin synthase 2* and *insect intestinal mucin* expression with ecdysone responsive transcription factors in *Helicoverpa armigera*. Insect Biochemistry and Molecular Biology 190:104521.

Jongejan F, Crafford D, Erasmus H, *et al*., 2016. Comparative efficacy of oral administrated afoxolaner (NexGard™) and fluralaner (Bravecto™) with topically applied permethrin/imidacloprid (Advantix(®)) against transmission of *Ehrlichia canis* by infected *Rhipicephalus sanguineus* ticks to dogs. Parasites & Vectors 9:348.

Labruna MB, Doretto JS, de Araújo Nascimento OC, *et al*., 2023. Efficacy of either orally administered fluralaner or topically administered imidacloprid/flumethrin for controlling *Rhipicephalus sanguineus* sensu lato premises infestations. Parasites & Vectors 16:414.

Laudani F, Campolo O, Caridi R, *et al*., 2022. Aphicidal activity and phytotoxicity of *Citrus sinensis* essential-oil-based nano-insecticide. Insects 13:1150.

Li C, Li Y, Li B, *et al*., 2026. Key cuticular proteins mediating molting and environmental stress response in *Panonychus citri*. Pest Management Science 82:4669–4678.

Li Y, Yu S, Huang J, *et al*., 2022a. Study of behavioral, electrophysiological response, and the active compounds of the essential oils from six kinds of flowers against *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Industrial Crops and Products 188:115603.

Li ZM, Robinson M and Kannan K, 2022b. An assessment of exposure to several classes of pesticides in pet dogs and cats from New York, United States. Environment International 169:107526.

Liu Z, 2022. Identification and analysis of jasmine essential oil by gas chromatography and mass sectrometry. Fine and Specialty Chemicals 30:36–40.

Mei R, Shi Y, Li X, *et al*., 2024. Detoxification mechanism of hinokitiol by *Alternaria alternata* and its application in agricultural antifungal control. Journal of Agricultural and Food Chemistry 72:21495–21502.

Moraga FA, Muñoz HC, Sánchez SM, *et al*., 2023. Exploring the diversity of tick-borne pathogens: The case of bacteria (*Anaplasma*, *Rickettsia*, *Coxiella* and *Borrelia*) protozoa (*Babesia* and *Theileria*) and viruses (*Orthonairovirus*, tick-borne encephalitis virus and louping ill virus) in the European continent. Veterinary Microbiology 286:109892.

Nwanade CF, Wang M, Pei T, *et al*., 2024. Toxicity and enzymatic mechanism of Citrus spp. essential oils and major constituents on *Haemaphysalis longicornis* (Acari: Ixodidae) and non-target *Harmonia axyridis* (Coleoptera: Coccinellidae). Pesticide Biochemistry and Physiology 204:106113.

Pazinato R, Volpato A, Baldissera MD, *et al*., 2016. *In vitro* effect of seven essential oils on the reproduction of the cattle tick *Rhipicephalus microplus*. Journal of Advanced Research 7:1029–1034.

Petretto GL, Vacca G, Addis R, *et al*., 2023. Waste *Citrus limon* leaves as source of essential oil rich in limonene and citral: chemical characterization, antimicrobial and antioxidant properties, and effects on cancer cell viability. Antioxidants (Basel, Switzerland) 12:1238.

Pfannenstiel LJ, Buchon N and Scott JG, 2026. The ABC transporter vitalporter (*CG3164*) is necessary for development and survival in *Drosophila melanogaster*. Journal of Insect Physiology 170:104973.

Prado-Rebolledo OF, Molina-Ochoa J, Lezama-Gutiérrez R, *et al*., 2017. Effect of metarhizium anisopliae (Ascomycete), cypermethrin, and d-limonene, alone and combined, on larval mortality of *Rhipicephalus sanguineus* (Acari: Ixodidae). Journal of Medical Entomology 54:1323–1327.

Quintana AJ, García-Suárez R, Prieto A, *et al*., 2026. Genome editing of ABCB6 transporter confers resistance to cypermethrin in the major pest of corn, *Spodoptera frugiperda*. Insect Biochemistry and Molecular Biology 190:104537.

Ren R, Zhao S, Jia H, *et al*., 2026. Maize *Chlorotic Leaf Spot1* encodes a fumarylacetoacetate hydrolase essential for carbohydrate partitioning. Journal of Genetics and Genomics 53:679–690.

Rosado AJA, Arjona CK, Torres AJFJ, *et al*., 2017. Plant products and secondary metabolites with acaricide activity against ticks. Veterinary Parasitology 238:66–76.

Singh N and Singh SK, 2016. *Citrus limon* extract: possible inhibitory mechanisms affecting testicular functions and fertility in male mice. Systems Biology in Reproductive Medicine 62:39–48.

Sterkel M and Oliveira PL, 2017. Developmental roles of tyrosine metabolism enzymes in the blood-sucking insect *Rhodnius prolixus*. Proceedings. Biological Sciences 284:20162607.

Ullah F, Ullah Z, Güncan A, *et al*., 2025. Tetraniliprole triggers transgenerational hormesis in an invasive insect herbivore: molecular and biological insights. Insects 16:1073.

Villanueva-Pereira T, Silva-Aguayo G, Rodríguez M, *et al*., 2025. Contact, fumigant and repellent activities of essential oils of cinnamon, lemon, and peppermint against larvae and adults of *Musca domestica* L. Chilean Journal of Agricultural Research 85:318–329.

Wang H, Li K, Li D, *et al*., 2026a. *Jasminum sambac*: progress in research on ethnopharmacology, phytochemistry, pharmacological actions and other applications. Journal of Ethnopharmacology 354:120478.

Wang H, Xin T, Jiang C, *et al*., 2026b. *PcCPR12* participates in coordinating chitin and lipid metabolism to maintain cuticle structural stability in *Panonychus citri*. Pest Management Science.

Wang M, Gong L, Wang Y, *et al*., 2025. Survey of hard ticks in high-incidence area of severe fever with thrombocytopenia syndrome in China. Experimental and Applied Acarology 96:5.

Wanna R, Khaengkhan P and Bunphan PBH, 2023. Insecticidal activity of essential oil from *Jasminum sambac* (L.) aiton against *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Bruchidae). Plant Protection Science 59:369–378.

Wood MJ, Yavaşoğlu SI, Bull JC, *et al*., 2026. A natural barrier: tick-repellent potential of a spruce-derived volatile blend against *Hyalomma excavatum* and *Ixodes ricinus*. Pest Management Science 82:1481–1491.

Yang X, Zhang S, Gao J, *et al*., 2025. Chemical composition and acaricidal properties of essential oils from *Cinnamomum mollifolium*, *Eucalyptus globulus*, and *Pimenta dioica* against *Hyalomma rufipes* (Acari: Ixodidae). BMC Veterinary Research 21:683.

Yuan L, Li T, Jiang D, *et al*., 2026. Molecular basis of polyphagy: the detoxification gene *HcGST18* underlies host plant adaptation in the *Hyphantria cunea*. Insect Science.

Zhang S, Gao Z, Dong K, *et al*., 2024b. Functional analysis of novel cystatins from *Haemaphysalis doenitzi* and evaluation of their roles in cypermethrin and λ-cyhalothrin resistance. Pesticide Biochemistry and Physiology 204, 106075.

Zhang S, Gao Z, Ghonaim AH, *et al*., 2025a. Cystatin antibodies interfere with ovary development in *Haemaphysalis doenitzi* (Acari: Ixodidae). PLoS Neglected Tropical Diseases 19:e0013064.

Zhang S, Gao Z, Guo J, *et al*., 2025c. Unveiling the potential: exploring the efficacy of *Coriandrum sativum* essential oil in *Haemaphysalis doenitzi* tick control. Pesticide Biochemistry and Physiology 215:106661.

Zhang S, Gao Z, Wang H, *et al*., 2025b. Acaricidal bioactivity and molecular target analysis of *Origanum onites* and *Ocimum gratissimum* essential oils against *Haemaphysalis doenitzi* ticks. Parasites & Vectors 18:401.

Zhang S, Gao Z, Yao M, *et al*., 2026. Tick control potential of *Thymus vulgaris* essential oil: Repellency, acaricidal activity, and enzyme-modulating effects against *Haemaphysalis doenitzi* (Acari: Ixodidae). Pakistan Veterinary Journal 46:1197–1208

Zhang SB, Gao ZH, Wang YK, *et al*., 2024a. The evaluation of cystatin protein vaccines based on the stress response of ticks triggered by low-temperature and toxin stress in *Haemaphysalis doenitzi*. Pest Management Science 80:3957–3966.