



Histopathological alterations in the synganglion of female *Rhipicephalus linnaei* ticks treated with essential oils of *Egletes viscosa* and *Lippia schaueriana*

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Abstract

The inappropriate use of synthetic chemical acaricides for tick management has contributed to the selection of acaricide-resistant tick strains, thereby stimulating the search for alternative and effective tick control strategies. Among these, the use of essential oils (EOs) derived from plants such as *Egletes viscosa* Less. (Asteraceae) and *Lippia schaueriana* Mart. (Verbenaceae) have shown promise in the control of insects and other arthropods. This study aimed to evaluate the effects of *E. viscosa* and *L. schaueriana* EOs on the morphohistology of the tick central nervous system (synganglion)—in *Rhipicephalus linnaei* semi-engorged females. The morphological alterations in the synganglion were assessed using histopathological and semi-quantitative analyses. The EOs, extracted via hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC–MS), revealed 27 constituents in *E. viscosa* and 18 in *L. schaueriana*, with cis-isopinocarveol acetate and piperitenone oxide identified as the predominant compounds, respectively. Histological and semi-quantitative analyses of the synganglion in EO-exposed females revealed dose-dependent alterations that were more severe than the mild vacuolization observed in the ethanol control group, primarily characterized by disruptions in the external membrane structures (neurilemma and perineurium), degeneration of the cortical region with hypertrophic, pyknotic, and fragmented neuronal nuclei, cytoplasmic vacuolization, and neuropil fiber disorganization. These findings suggest that the essential oils of *E. viscosa* and *L. schaueriana* induce histopathological damage in the synganglion, including neuronal cell damage and potentially impairing other central nervous system-mediated functions, such as feeding and reproduction. This supports the potential of these oils as candidates for the development of novel plant-based acaricides.

Keywords Ectoparasites · Botanical acaricides · *Egletes viscosa* · *Lippia schaueriana* · Synganglion

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Introduction

Ticks are ectoparasites with a global distribution and are recognized vectors of pathogens that impact human and animal health (Amrutha et al. 2021). These pathogens are responsible for tick-borne diseases (TBDs), which are often severe and increasing in incidence, thereby posing significant threats to public and veterinary health (Galay et al. 2018).

The *Rhipicephalus linnaei* (Audouin, 1826)—formerly referred to as the tropical lineage of *R. sanguineus* sensu lato (Šlapeta et al. 2022)—is a tick species found across the Americas, Africa, Europe, Asia, and Australia (Dantas-Torres et al. 2024; Šlapeta et al. 2022). This distribution allows

the species to parasitize not only its primary host, the domestic dog, but also other animals including humans (Camargo-Mathias 2018; Dantas-Torres et al. 2024; Sonenshine and Roe 2014). This species is associated with the transmission of several pathogens of medical and veterinary importance, including *Ehrlichia canis*, *Babesia* spp., *Hepatozoon canis* and *Anaplasma platys* (Takhampunya et al. 2025).

The threat of disease transmission by ticks has resulted in an increasing use of synthetic chemical acaricides that can be applied to companion animals to potentially reduce pathogen transmission and TBD incidence (Galay et al. 2018). However, widespread use of these products has contributed to the selection of resistant strains, particularly against pyrethroid compounds (Godara et al. 2019; Wyk et al. 2016; Kumar et al. 2020; Stone et al. 2024; Tian et al. 2023). Recent evidence of phenotypic permethrin resistance associated with mutations in the voltage-gated sodium channel gene in *Rhipicephalus linnaei* populations directly supports the relevance of investigating alternative control strategies for this species (Takhampunya et al. 2025).

Plant-derived bioactive compounds have been investigated as alternatives for tick control (Luns et al. 2021). Essential oils are particularly promising because they contain complex mixtures of active constituents that may act through different biological pathways against arthropods (Matos et al. 2014; Anholetto et al. 2017). Among these plants, *Egletes viscosa* (L.) Less. (Asteraceae), popularly known as “macela” or “macela-da-terra”, is native to tropical America, including northeastern Brazil, and has been associated with pharmacological and bioactive properties (Souza et al. 1992; Lorenzi and Mattos 2008; Pereira et al. 2018). *Lippia schaueriana* Mart. (Verbenaceae), known as “alecrim-de-moço” or “lipia-da-serra”, is endemic to the Brazilian Caatinga and occurs mainly in northeastern Brazil (Lorenzi and Mattos 2008; Souza et al. 2018). The essential oils of these species are characterized mainly by cis-isopinocarveyl acetate in *E. viscosa* and piperitenone oxide in *L. schaueriana*, and their effects on tick reproductive biology have been previously reported (Pereira et al. 2023).

Beyond acaricidal activity, sublethal exposure of various tick species to plant-derived bioactives has been associated with morphophysiological damage to essential organs, including salivary glands, ovaries, the Gené's organ, and the synganglion (tick central nervous system (CNS)) (Anholetto et al. 2018; Camargo-Mathias 2018; Camargo-Mathias et al. 2024; König et al. 2021; Lima de Souza et al. 2019; Matos et al. 2019; Remedio et al. 2014). Owing to its central role in tick biology, the synganglion is frequently targeted by acaricides, whose primary mode of action involves the disruption of neurotransmitter synthesis and consequent organ dysfunction (Pereira et al. 2017; Roma et al. 2014).

In this sense, we assessed here the first report on the action of different concentrations of EOs from *E. viscosa* and *L. schaueriana* on the synganglion of semi-engorged *R. linnaei* females.

Material and methods

Ticks

Unfed male and female ticks were obtained from laboratory colonies maintained by the Brazilian Central of Studies on Ticks Morphology (BCSTM) at the Animal Facility of the Institute of Biosciences, São Paulo State University “Júlio de Mesquita Filho” (UNESP), Rio Claro campus, São Paulo, Brazil. Ticks were kept in an incubator (Eletrolab EL 202) under controlled conditions (29 °C, 80% relative humidity, and a 12 h light/dark photoperiod) prior to infestation.

Partially engorged *R. linnaei* females were obtained through artificial infestation on naïve rabbit hosts (Genetic Group of Botucatu) that had no previous contact with ticks or essential oils. The rabbits were housed in restraining cages within a dedicated experimental room at the same facility and had free access to food and water throughout the experiment period.

For each infestation, 50 unfed females and 50 unfed males were placed into feeding chambers attached to the dorsal region of the host animals. Eight hours after tick placement, we inspected the attachment sites of the females and conducted daily observations until day six of engorgement, when we collected the females.

The feeding chamber construction, infestation procedures, and tick handling followed the Bechara et al. (1995) protocol, in accordance with the Ethics Committee for Animal Use (CEUA-IB-UNESP/Rio Claro), protocol no. 17/2019.

Essential oils (EOs)

Dried flower heads of *E. viscosa* were purchased from a local market in Fortaleza (Ceará, Brazil) in March 2016. The extraction and analysis were conducted at the Embrapa Tropical Agroindustry Research Unit in Fortaleza, Ceará, Brazil. For oil extraction, 300 g of capitula were mixed with 1.5 L of distilled water in a 3 L round-bottom flask and subjected to hydrodistillation for 4 h using a Clevenger-type apparatus. The essential oil (yield: 1.2%, v/w) was collected, centrifuged (2000 RPM; 10 min), dried with anhydrous sodium sulphate, and stored in amber vials at – 20 °C for subsequent gas chromatography-mass spectrometry (GC–MS) and flame ionization detection (GC–FID) analysis.

Specimens of *L. schaueriana* were collected in March 2015 (8°22'58.2"S, 42°18'31.4"W) in São João do Piauí, Piauí, Brazil. A reference sample was deposited in the Leopoldo Krieger Herbarium (CESJ) at the Federal University of Juiz de Fora, in Juiz de Fora, Minas Gerais, Brazil, under voucher number 67.395. Oil extraction was carried out at the Embrapa Meio-Norte Research Unit in Parnaíba, Piauí, Brazil. Approximately 2 kg of fresh leaves were mixed with 3 L of water and subjected to hydrodistillation for 3 h using a Clevenger apparatus. The essential oil (yield: 1.2%, v/w) was collected, centrifuged (2000 RPM; 10 min), dried with anhydrous sodium sulphate, and stored in amber vials at – 20 °C. GC–MS and GC-FID analyses were conducted at Embrapa Tropical Agroindustry Research Unit in Fortaleza, Ceará, Brazil.

Adult immersion test

Prior to testing, partially engorged females were assigned to eight experimental groups (10 specimens per concentration, totalling 80 engorged females), ensuring homogeneous weight through analytical balance measurement. Specimens were washed with running water using a sieve, then dried with soft absorbent paper.

The experimental groups were as follows:

- Control Group 1 (C1): females exposed to distilled water only;
- Control Group 2 (C2): females exposed to the oil solvent (50% ethanol, v/v);
- Treatment Groups (T1–T6): females exposed to essential oils at the following concentrations:
 - T1: *E. viscosa* EO at 12.5 mg/mL
 - T2: *E. viscosa* EO at 25 mg/mL
 - T3: *E. viscosa* EO at 50 mg/mL
 - T4: *L. schaueriana* EO at 12.5 mg/mL
 - T5: *L. schaueriana* EO at 25 mg/mL
 - T6: *L. schaueriana* EO at 50 mg/mL

Each group of females was immersed in the respective solution (EOs, distilled water, or ethanol 50%) for 5 min (Drummond et al. 1973). After immersion, ticks were dried on absorbent paper and placed in Petri dishes under controlled conditions (28 ± 1 °C, 80% humidity, 12 h photoperiod) in an incubator for 5 days, allowing for observation of morphological effects which are typically delayed following acaricidal exposure.

The concentrations tested were selected based on a previous study using the same essential oils, in which 12.5, 25, and 50 mg/mL induced concentration-dependent effects on reproductive parameters and control efficacy in

Rhipicephalus sanguineus sensu lato females (Pereira et al. 2023).

Morphohistological analysis

The females were dissected in saline solution (NaCl 7.5 g/L, Na₂HPO₄ 2.38 g/L, KH₂PO₄ 2.72 g/L) to remove the dorsal shield and expose internal organs. The intestine—occupying most of the body cavity—was discarded, and to ensure optimal preservation of the synganglion, whole specimens were fixed in 4% paraformaldehyde as described by Pereira et al. (2017). After 7 days, the samples were transferred to phosphate buffer for 24 h, dehydrated in a graded ethanol series (70%, 80%, 90%, and 95%; 30 min per bath), and embedded in Leica® historesin for 7 days. The samples were then mounted in plastic molds with resin and hardener for an additional 7 days.

The resin blocks were sectioned at 3 µm using a LEICA® RM 2255 microtome. Sections were placed on pre-cleaned glass slides and stained with Harris hematoxylin (Sigma Aldrich, St. Louis, Missouri, USA) (10 min), rinsed in running water (5 min), counterstained with aqueous eosin (Sigma Aldrich, St. Louis, Missouri, USA) (5 min), and washed again. Slides were air-dried, mounted in Entellan® (Sigma Aldrich, St. Louis, Missouri, USA) with coverslips, and examined and photographed using a LEICA® DM750 brightfield light microscope.

Semi-quantitative analysis

Morphological alterations were assessed semi-quantitatively according to the method described by Marinho et al. (2014) and Konig et al. (2019), enabling statistical evaluation of the extent and impact of damage caused by EO exposure. An importance factor (ω) ranging from 1 to 3 was assigned to each observed alteration based on its relevance to synganglion function and on the expected severity and functional impact of the lesion on synganglion integrity: (1) minimal importance – alteration potentially reversible upon cessation of exposure; (2) moderate importance – alteration reversible in most cases; (3) maximum importance – irreversible lesion, according to the degree of tissue damage and potential impairment of organ function.

Alterations were also scored (α) on a scale from 0 to 5, where 0 represented normal morphology (similar to controls) and 5 indicated alterations in all observed cells/structures. The total index was calculated using the equation: $\text{Index}_{\text{ind}} = \sum(\omega \times \alpha)$. Because the semi-quantitative scores represent ordinal data, differences among groups were analyzed using the Kruskal–Wallis test followed by Dunn's multiple comparisons test ($p < 0.05$), using GraphPad Prism software (version 8.00).

Table 1 Chemical composition of *Egletes viscosa* Less. and *Lippia schaueriana* Mart. essential oils

Compounds	RI ^a	RI _{lit} ^b	Relative abundance (%)	
			<i>E. viscosa</i>	<i>L. schaueriana</i>
α -Thujene	928	928	0.18	—
α -Pinene	937	936	0.37	0.38
Sabinene	976	973	0.66	0.04
β -Pinene	981	978	5.16	—
Myrcene	992	989	0.36	0.71
α -Terpinene	1019	1017	0.12	—
ρ -Cymene	1026	1024	0.11	0.71
Limonene	1030	1030	0.67	21.29
<i>trans</i> - β Ocimene	1054	1050	—	0.78
γ -Terpinene	1059	1060	0.22	0.28
1-Terpineol	1137	1136	0.40	—
<i>trans</i> -Pinocarveol	1141	1140	0.55	—
<i>cis</i> -p-mentha-2,8-dien-1-ol	1145	1137	—	0.60
<i>cis</i> -Chrysanthanol	1162	1164	0.14	—
Terpinen-4-ol	1179	1177	0.47	—
α -Terpineol	1192	1190	0.42	—
Myrtenol	1198	1194	0.20	—
Methyl chavicol	1206	1196	—	0.54
<i>trans</i> -Carveol	1212	1217	2.70	—
Coahuilensol, methylether	1226	1221	—	0.55
Car-3-en-2-one	1244	1248	0.19	—
Linalool acetate	1251	1255	0.11	—
Carvone	1255	1243	—	0.35
<i>cis</i> -Chrysanthenyl acetate	1262	1262	4.22	—
Thymol	1298	1290	—	1.64
<i>trans</i> -pinocarveyl acetate	1302	1298	10.33	—
<i>cis</i> -isopinocarveyl acetate	1312	1305	68.41	—
Myrtenol acetate	1327	1329	1.01	—
<i>trans</i> -carvyl acetate	1345	1338	—	0.34
Piperitenone	1355	1343	—	2.83
Linalool isobutyrate	1371	1375	0.55	—
Piperitenone oxide	1383	1368	—	64.36
Butyl propanoate	1383	1384	0.12	—
<i>trans</i> -Myrtenol acetate	1386	1386	0.21	—
4 α ,7 α ,7 α -Nepetalactone	1414	—	—	3.30
<i>E</i> -Caryophyllene	1422	1420	0.47	—
Bicyclogermacrene	1510	1500	—	0.36
Caryophyllene oxide	1585	1581	0.27	—
Spathulenol	1593	1578	—	0.90
Total			98.62	99.96

^a: Retention index (RI) obtained by HP-5 MS; ^b: References (RI_{lit}) (ADAMS, 2017; BABUSHOK; LINSTROM; ZENKEVICH, 2011; CRAVEIRO et al. 1992; CUNHA, 2003). (—) Not identified. Bold values indicate the compounds with the highest relative abundance in each essential oil

Each female tick was considered an independent biological replicate, since the synganglion from each specimen was individually processed, analyzed, and scored.

Results

Chemical composition of the essential oils

The chemical characterization of the essential oils from *Egletes viscosa* and *Lippia schaueriana* identified 27 and 18 compounds, respectively, comprising over 98% of the constituents in both oils. The complete list of compounds, including the major ones, is presented in Table 1.

Morphology of the Synganglion of *R. linnaei*

Control groups

In females allocated to Control Group 1 (exposed to distilled water), the morphology of the synganglion was preserved, as expected for the organ under normal conditions. The enveloping membranes were intact, with an acellular neurilemma forming the outermost layer of the organ and the perineurium—composed of glial cells—located between the neurilemma and the cortex (Fig. 1A, A2). The cortical cells and the fibrous extensions forming the neuropil were also preserved (Fig. 1A1, A2). The intact subperineurium, composed of glial cells, was identified between the cortex and the neuropil (Fig. 1A1). Figure 1A also shows the esophageal canal traversing the organ.

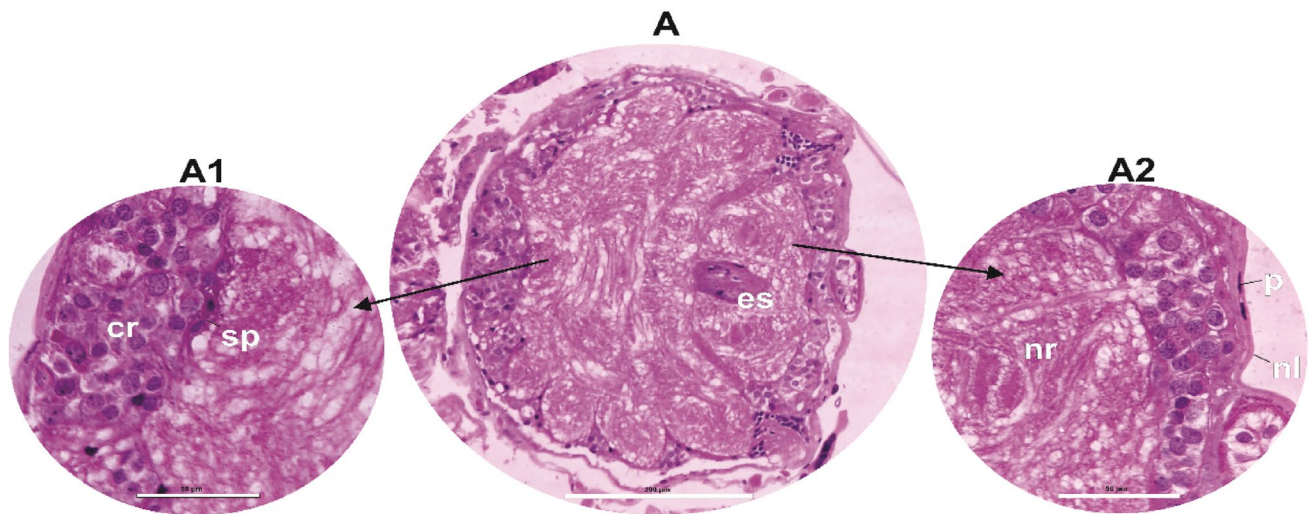
In females from Control Group 2 (exposed to 50% ethanol), in addition to the characteristics described for Control 1, morphological changes were identified in the cortex and neuropil, such as cytoplasmic vacuolization (Fig. 1B, B2).

Exposure of females to *Egletes viscosa* oil (treatment groups 1–3)

The essential oil of *E. viscosa* induced dose-dependent histopathological alterations in the synganglion of semi-engorged *R. linnaei* females. In Group 1 (exposed to 12.5 mg/mL of *E. viscosa* oil), some morphological changes were observed in the organ, including detachment of the neurilemma and perineurium, which was statistically significant ($p < 0.05$) compared to controls in the semi-quantitative analysis (Table 2), along with cytoplasmic vacuolization in cortical cells (Fig. 2A, A2).

In Group 2 (25 mg/mL), the previously mentioned changes intensified. Detachment of both the neurilemma and perineurium was observed in various regions, along with folds and irregularities in both membranes (Fig. 2 B,

Control 1



Control 2

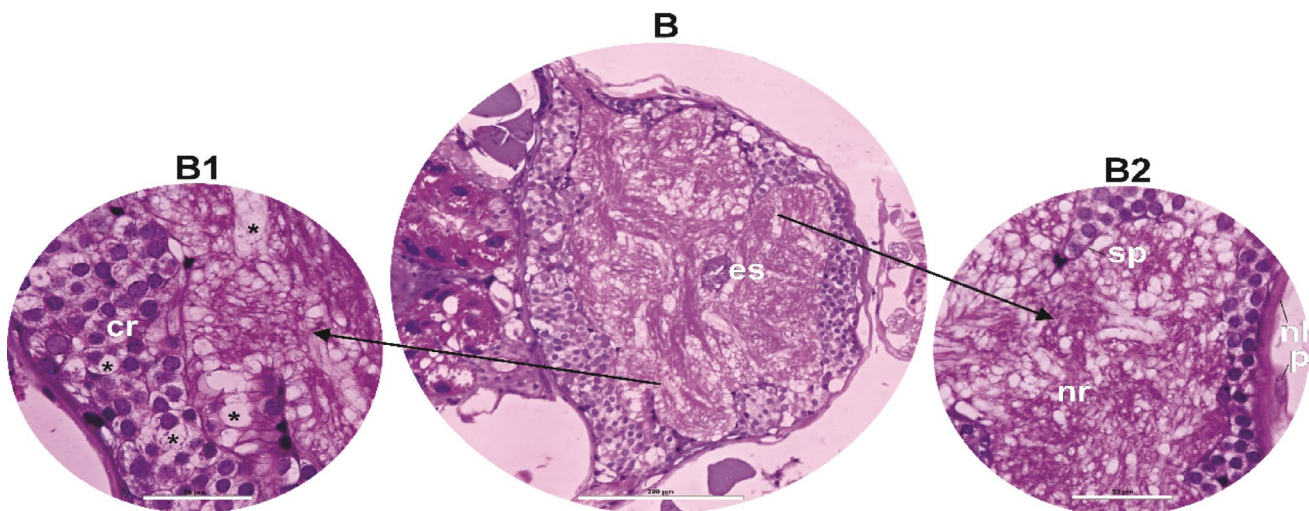


Fig. 1 Histological sections of the synganglion of *Rhipicephalus linnaei* semi-engorged female from Control Group 1 (distilled water) (**A**, **A2**) and Control Group 2 (ethanol 50%) (**B**, **B2**) stained with hema-

toxylin–eosin (HE). cr: cortex, es: esophagus, nl: neurilemma, nr: neuropil, p: perineurium, sp: subperineurium, asterisk (*): vacuolization. Bars: (**A**, **B**): 200 μ m; (**A1–B2**): 50 μ m

B2). Several cortical cells showed hypertrophic and pyknotic nuclei—indicative of cell death. The subperineurium was also ruptured in some areas (Fig. 2B1, B2). All morphological alterations in this group were statistically significant ($p < 0.05$) (Table 2).

Group 3 females (50 mg/mL) exhibited the most severe morphological damage. The enveloping membranes

(neurilemma and perineurium) and the subperineurium were almost completely ruptured (Fig. 2C, C1). This disruption allowed cortical cells to invade the neuropil region, making it impossible to distinguish between the cortex and the neuropil (Fig. 2C, C2). Cortical tissue also showed cells at various stages of apoptotic death, including pyknotic, hypertrophic nuclei, and nuclear fragmentation (Fig. 2C1,

Table 2 Occurrence of morphological alterations in the synganglion of female *R. linnaei* exposed to essential oils of *E. viscosa* and *L. schaueriana*

	Morphological alterations	(ω)	Control 1	Control 2	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6
Neurilemma e Perineurium	Membrane folding and irregularities	1	0.0±0.0	0.0±0.0	0.40±0.54	1.60±0.54*	3.80±0.83*	0.60±0.54	1.00±0.70*	4.20±0.44*
	Detachment from the organ	2	0.0±0.0	0.0±0.0	1.60±0.89*	2.00±1.41*	6.80±1.09*	0.60±0.54	1.00±0.70	4.20±0.45*
	Membrane rupture	2	0.0±0.0	0.0±0.0	0.0±0.0	2.00±1.41*	5.60±1.67*	0.0±0.0	2.50±1.00*	5.20±1.09*
Subperineurium	Membrane rupture	2	0.0±0.0	0.0±0.0	0.40±0.89	1.60±0.89*	8.80±1.09*	0.40±0.89	2.40±0.89*	9.20±1.09*
	Loss of glial cell integrity	2	0.0±0.0	0.0±0.0	0.0±0.0	0.80±1.09	8.80±1.09*	0.0±0.0	0.80±1.09	8.40±1.67*
Neuropil	Cytoplasmic vacuolization	1	0.0±0.0	0.20±0.44	0.40±0.54	1.60±0.54*	3.60±0.89*	0.60±0.54	1.40±0.54*	4.40±0.54*
	Disorganization of fibers	2	0.0±0.0	0.0±0.0	0.40±0.89	2.80±1.09*	7.20±1.09*	0.40±0.89	2.40±1.67*	7.60±1.67*
	Loss of tissue integrity	3	0.0±0.0	0.0±0.0	0.0±0.0	4.80±1.64*	12.60±1.34*	0.0±0.0	4.80±1.64*	13.20±1.64*
Cortex	Cytoplasmic vacuolization	1	0.0±0.0	0.20±0.44	0.60±0.54	1.80±0.44*	3.40±0.54*	0.40±0.54	1.60±0.54*	3.60±0.54*
	Cellular disorganization	2	0.0±0.0	0.0±0.0	0.40±0.89	4.00±0.0*	7.60±1.67*	0.40±0.89	3.60±0.89*	9.60±0.89*
	Loss of tissue integrity	3	0.0±0.0	0.0±0.0	0.60±1.34	5.40±1.34*	10.80±1.64*	1.20±1.64	6.00±0.0*	14.40±1.34*
Neuronal cells	Hypertrophy	3	0.0±0.0	0.0±0.0	1.80±2.68	4.80±1.64*	10.20±1.64*	1.80±1.64	5.40±1.34*	9.60±1.34*
	Nuclear pyknosis	3	0.0±0.0	0.0±0.0	2.40±2.51	4.80±1.64*	11.40±1.34*	1.80±1.64	6.0±0.0*	10.80±1.64*
	Nuclear fragmentation	3	0.0±0.0	0.0±0.0	0.0±0.0	1.20±1.64	11.40±1.34*	0.0±0.0	1.80±1.64	12.60±2.51*
	Intracytoplasmic vacuolization	1	0.0±0.0	0.0±0.0	0.60±0.54	1.20±0.83*	3.0±0.0*	0.60±0.54	1.60±0.54*	3.0±0.70*
	Loss of cellular integrity and boundaries	3	0.0±0.0	0.0±0.0	0.60±1.34	3.60±1.34*	10.80±1.64*	0.60±1.34	4.20±1.64*	11.40±2.51*

(ω): importance factor; Control 1: distilled water; Control 2: ethanol 50%; Treatment 1: 12.5 mg/mL *E. viscosa*; Treatment 2: 25 mg/mL *E. viscosa*; Treatment 3: 50 mg/mL *E. viscosa*; Treatment 4: 12.5 mg/mL *L. schaueriana*; Treatment 5: 25 mg/mL *L. schaueriana*; Treatment 6: 50 mg/mL *L. schaueriana*.

Values represent mean±SD. Statistical analysis was performed using the Kruskal–Wallis test followed by Dunn's multiple comparisons test. (*) $p<0.05$ compared with Control Group 1

C2). The changes were statistically significant in the semi-quantitative analysis ($p<0.05$) (Table 2).

Exposure of females to *Lippia schaueriana* oil (treatment groups 4–6)

In Group 4 (12.5 mg/mL), the synganglion displayed some morphological changes similar to those observed in Treatment Group 1. Although not statistically significant (Table 2), cytoplasmic vacuolization in the cortex and neuropil, as well as detachment of external membranes in some regions, were observed (Fig. 2D). The neurilemma and perineurium showed folds and irregularities (Fig. 2D, D2). Most cortical nuclei remained intact, with only a few showing hypertrophy and pyknosis (Fig. 2D1, D2).

In Group 5 (25 mg/mL), more severe alterations occurred, including detachment and rupture of the neurilemma and perineurium. Cortical cells displayed typical apoptotic features such as nuclear pyknosis and hypertrophy (Fig. 2E, E2). The subperineurium showed some irregularities (Fig. 2E1). These changes were statistically significant ($p<0.05$) (Table 2).

The most severe damage occurred in Group 6 (50 mg/mL), where both the neurilemma and perineurium lost their original organization and were ruptured (Fig. 2F, F2). The cortex, subperineurium, and neuropil could no longer be distinguished (Fig. 2F). A large vacuole formed between the external membranes and the few remaining cortical cells and fibrous neuropil extensions. This vacuole contained numerous cellular and cytoplasmic fragments (Fig. 2F-F2).

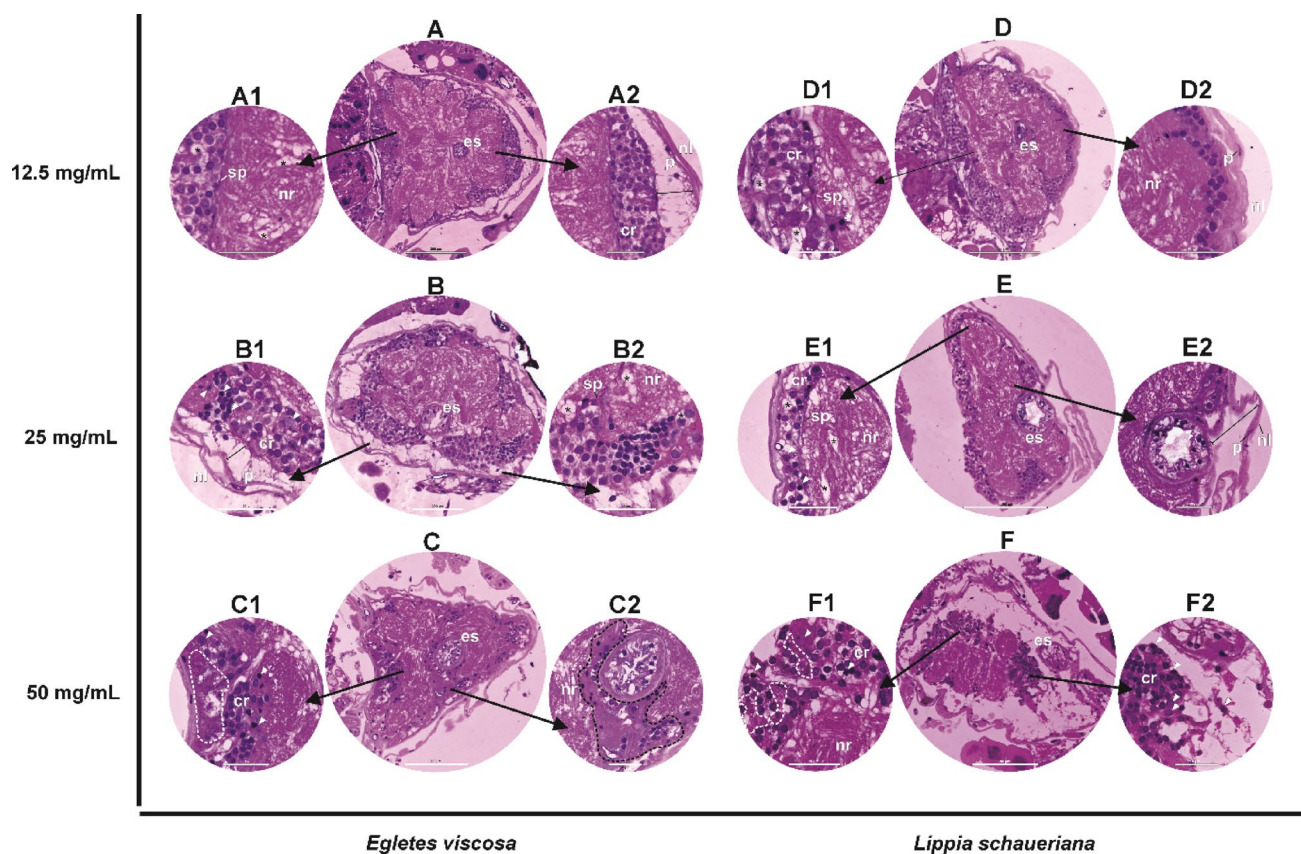


Fig. 2 Histological sections of the synganglion of *Rhipicephalus linnaei* semi-engorged female from the Treatment Groups, exposed to different concentrations of essential oils from *Egletes viscosa* and *Lippia schaueriana*, and stained with HE. **A–A2:** 12.5 mg/mL of *E. viscosa*; **B, B2:** 25 mg/mL of *E. viscosa*; **C, C2:** 50 mg/mL of *E. viscosa*; **D, D2:** 12.5 mg/mL of *L. schaueriana*; **E, E2:** 25 mg/mL of *L. schaueriana*; **F, F2:** 50 mg/mL of *L. schaueriana*. cr: cortex, es: esophagus, nl: neurilemma, nr: neuropil, p: perineurium, sp: subperineurium, asterisk (*): vacuolization, White arrow: characteristics of apoptotic death (pyknosis and/or nuclear hypertrophy), white highlighted area: nuclear fragmentation, black highlighted area: neuropil invasion by cortical cells. Bars: (A–F): 200 µm; (A1–F2): 50 µm

With the disruption of the subperineurium, cortical cells—many in apoptotic stages—intermingled with the neuropil extensions (Fig. 2F1, F2). The semi-quantitative analysis confirmed that the observed alterations were statistically significant compared to controls ($p < 0.05$) (Table 2).

Discussion

We investigated the effects of essential oils from *Egletes viscosa* and *Lippia schaueriana* on the central nervous system of semi-engorged *Rhipicephalus linnaei* females, assessing the extent of damage to this vital organ for tick survival and reproduction success.

Studies evaluating the potential of essential oils extracted from plants have been increasingly developed, and the results demonstrate their broad range of applications. Specifically, the essential oils (EOs) extracted from *Egletes viscosa* Less. and *Lippia schaueriana* Mart. have already had their diverse biological activities reported in the literature,

including insecticidal and acaricidal effects (Oliveira et al. 2020; Pereira et al. 2023; Singh and Maurya 2005). Although these results are promising, little is still known about the mode of action of these oils on the morphophysiology of animals exposed to them, especially ticks.

The chemical analysis showed that the EO of *E. viscosa* contains 27 components, with cis-isopinocarveyl acetate as the major constituent, confirming that the oil used here was extracted from chemotype B of this plant and corroborating other chemical composition studies of this species (Pereira et al. 2023; Carvalho et al. 2021; Vieira et al. 2006). In earlier studies, Vieira et al. (2006) identified two chemotypes within the *E. viscosa* species, whose distinction was primarily based on the major component: plants with trans-pinocarveyl acetate belong to chemotype A, the first to be described, while those with cis-isopinocarveyl acetate, as in this study, belong to chemotype B.

The EO of *L. schaueriana* was found to contain 18 components, with piperitenone oxide being the major constituent, in agreement with findings by Souza et al. (2018) and

Pereira et al. (2023). Although this mechanism has not yet been confirmed in ticks, piperitenone oxide has been reported to inhibit acetylcholinesterase activity in insects, an enzyme involved in acetylcholine regulation and nerve impulse transmission (Miyazawa et al. 1998). Therefore, the severe alterations observed in the synganglion of females exposed to *L. schaueriana* EO may be related to the bioactive properties of this compound; however, further biochemical assays are required to confirm its mechanism of action in *R. linnaei*.

Females from the Control Group 1, exposed only to distilled water, showed no morphological alterations in the synganglion, which remained intact and consistent with the description provided by Roma et al. (2012) for this species. In Control Group 2, exposed to 50% ethanol, mild cytoplasmic vacuolization was observed in the cortex and neuropil. However, these alterations were limited when compared with the severe and dose-dependent damage observed in the EO-treated groups, particularly the disruption of the neurilemma, perineurium, and subperineurium, as well as nuclear alterations and neuropil disorganization. This finding supports the hypothesis that the severe morphological changes observed in the treatment groups were due to EO exposure.

It is important to consider that the toxicity of essential oils may vary considerably depending on their chemical composition, major constituents, concentration, exposure method, and the tick species or developmental stage evaluated. Therefore, although some plant-derived compounds have been reported to show low toxicity or variable effects under specific experimental conditions, the present findings indicate marked histopathological alterations in the synganglion under the concentrations and exposure protocol used here.

Both EOs showed dose-dependent damage to the synganglion of *R. linnaei* ticks. Morphological changes were evident even at the lowest concentrations tested (12.5 and 25 mg/mL). These included separation, folding, irregularities, and/or rupture of the outer membranes of the synganglion—the neurilemma and perineurium—supporting previous findings in ticks exposed to synthetic acaricides such as permethrin and deltamethrin (Pereira et al. 2017; Roma et al. 2013), as well as natural compounds like thymol (Matos et al. 2018). These results suggest that the outer membranes, which surround the synganglion, may act as selective barriers preventing toxic substances from entering via the hemolymph. Since these membranes are the first to come into contact with the acaricides, they are also the first to exhibit damage, even at the lowest concentration (12.5 mg/mL) of both *E. viscosa* and *L. schaueriana* EOs. Furthermore, according to Sonenshine and Roe (2014), the glial cells in the perineurium are responsible for the metabolic regulation of neuronal activity, and any structural

alteration to this membrane—such as those described in this study following EO exposure—can result in widespread dysfunction of the central nervous system.

The present findings also revealed that, except for Treatment Group 1 (12.5 mg/mL *E. viscosa* EO), all other EO-treated groups exhibited cortical degeneration in the synganglion, particularly in the neuronal cell nuclei, which appeared hypertrophic, pyknotic, and often fragmented—indicative of apoptosis. These results corroborate those previously reported for the synganglion of *R. linnaei* exposed to various acaricides (Pereira et al. 2017; Roma et al. 2013).

Intracytoplasmic vacuolization was observed in the cortical region of the synganglion in all exposed groups. This finding may indicate a cellular defence mechanism against the toxic compounds present in the essential oils. The sequestration of cytoplasmic debris and/or damaged organelles within vacuoles may represent an attempt by the cell to preserve undamaged regions. This same defence mechanism has been observed in other studies evaluating the harmful effects of acaricides on the CNS and other organs in *R. linnaei*, such as salivary glands (Furquim et al. 2008; Matos et al. 2018; Pereira et al. 2023; Roma et al. 2013).

Moreover, in all groups exposed to *E. viscosa* and *L. schaueriana* EOs, spaces were observed in the extracellular matrix between the fibrous extensions of the neuropil. This region is composed of lobes containing dendrites and axons of neuronal cells, and according to Pereira et al. (2017), alterations in the organization of these fibers, such as the vacuolization observed here, may result from damage inflicted on cortical cells by neurotoxic agents.

Ticks exposed to 50 mg/mL *L. schaueriana* EO, in addition to a loss of the organ's original structure—with the cortex and neuropil no longer distinguishable—numerous cellular and cytoplasmic fragments were observed in the large space formed between the cortex and peripheral membranes. This suggests that the space may have formed not only due to membrane separation but also as a result of intense degeneration in the cortical and neuropil regions. It is known that piperitenone oxide, the major compound in *L. schaueriana* EO, has neurotoxic effects in insects (Miyazawa et al. 1998), and the severe degeneration observed here may indicate that it acts similarly in ticks.

In Treatment Group 3, females exposed to 50 mg/mL *E. viscosa* EO also exhibited alterations, comparable to those in Treatment Group 6, with loss of the organ's original architecture. Advanced neuronal death in the cortex, as well as subperineurium disruption that allowed cortical cell invasion into the neuropil, likely impaired CNS function. Since the cortical region houses neurosecretory centers responsible for synthesizing and secreting regulatory substances (Sonenshine and Roe 2014), its damage would compromise these essential functions. This cortical invasion into

the neuropil following subperineurium destruction has also been reported by Pereira et al. (2017) in *R. linnaei* females exposed to the synthetic acaricide deltamethrin. These alterations suggest that *E. viscosa* EO may also act as a neurotoxin in ticks.

The structural disorganization of nervous tissue observed at 50 mg/mL EO concentration for both *E. viscosa* and *L. schaueriana* suggests a strong acaricide potential of these oils, possibly affecting other vital systems. Considering the CNS controls and regulates all systemic functions in ticks (Sonenshine and Roe 2014) such as reproduction and feeding, any disruption in synganglion morphophysiology would prevent the animal from successfully performing activities critical to survival.

Thus, based on all alterations observed in the synganglion of females exposed to *E. viscosa* and *L. schaueriana* EOs, it is concluded that these oils may act as potential acaricides, inducing cell death in nervous tissue—a vital organ for the success and survival of these ectoparasites.

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Author contributions M.C.P. was responsible for conceptualization, methodology, investigation, formal analysis, data curation, visualization, and writing of the original draft. L.A.A. contributed to resources, methodology, validation, and writing—review and editing. M.R.A. contributed to investigation, data curation, and writing—review and editing. K.N.C.C. contributed to resources, validation, and writing—review and editing. K.M.C. contributed to resources, software, methodology, and writing—review and editing. A.S.Q.S. contributed to resources and methodology. M.I.C.-M. contributed to supervision, project administration, conceptualization, methodology, validation, funding acquisition, and writing—review and editing. All authors reviewed and approved the final manuscript.

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Data availability All the data produced in this study are available at the link <https://figshare.com/s/834af8d9a1720700df21>, with <https://doi.org/10.6084/m9.figshare.30639065>.

Declarations

Conflicts of interest The authors declare that there are no conflicts of interest.

Human or animal rights All procedures involving animals were conducted in accordance with national and institutional guidelines for the care and use of laboratory animals. The experimental protocols were approved by the Ethics Committee for Animal Use of the Institute of Biosciences, São Paulo State University “Júlio de Mesquita Filho” (UNESP), Rio Claro campus (CEUA-IB-UNESP/Rio Claro, protocol no. 17/2019). All efforts were made to minimize animal suffering and to reduce the number of animals used.

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