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Chemical Composition and In Vitro Acaricidal Activity of *Schinus terebinthifolius* Essential Oils from Leaves and Fruits Against *Rhipicephalus microplus*

Eduardo Henrique Amorim Silva [1,2], Eduardo Fernandes Silva [1], Ananda Maria Freitas Freire Leão [1], Cicera Maria de Oliveira Xavier [1,2], Renata Silva Brito [1], Tatiene Rossana Móta Silva [1], Lucia Oliveira Macedo [1], Pedro Gregório Vieira Aquino [1], Gílcia Aparecida de Carvalho [1,2,3] and Rafael Antonio Nascimento Ramos [1,2,4,*]

[1] Laboratory of Parasitology, Federal University of the Agreste of Pernambuco, Garanhuns 55292-278, Pernambuco, Brazil; eduardo.amorimsilva@ufrpe.br (E.H.A.S.); eduardo.fernandessilva@ufape.edu.br (E.F.S.); nndleao@gmail.com (A.M.F.F.L.); ciceramox@gmail.com (C.M.d.O.X.); renatabrito8777@gmail.com (R.S.B.); tatienerms79@gmail.com (T.R.M.S.); lucia.macedo@ufape.edu.br (L.O.M.); pedro.aquino@ufape.edu.br (P.G.V.A.); gilcia.carvalho@ufrpe.br (G.A.d.C.)

[2] Graduate Program in Animal Bioscience, Federal Rural University of Pernambuco, Recife 52171-900, Pernambuco, Brazil

[3] Department of Biology, Federal Rural University of Pernambuco, Recife 52171-900, Pernambuco, Brazil

[4] Department of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, TX 77843, USA

* Correspondence: rafael.ramos@ufape.edu.br

Abstract

Resistance of ticks to synthetic acaricides has reduced the effectiveness of commercial products and increased interest in sustainable alternatives such as essential oils (EOs). This study characterized the chemical composition of EOs obtained from leaves and fruits of *Schinus terebinthifolius*, evaluated the in vitro acaricidal activity of EOs against *R. microplus*, and assessed its susceptibility to conventional acaricides. Ticks were collected from naturally infested cattle that had not been subjected to acaricidal treatment. EOs were obtained by hydrodistillation and characterized by gas chromatography–mass spectrometry. Adult immersion tests were performed in triplicate using seven combinations of synthetic acaricides, EOs at concentrations of 1%, 0.5%, and 0.25%, and control groups. The leaves and fruit EOs contained 48 and 20 compounds, respectively. Among the commercial acaricides, only two formulations achieved efficacy higher than 95%. The EOs exhibited concentration-dependent activity. At 1%, adult female mortality was 16.67% and 20.0% for leaf and fruit EOs, respectively, while the corresponding acaricidal efficacies were 96.69% and 97.90%. EO concentration showed positive and negative linear associations with oviposition inhibition and reproductive parameters, respectively. These findings indicate that the EOs of *S. terebinthifolius* exhibit in vitro acaricidal activity against *R. microplus*, supporting further investigation of their potential for tick control.

Keywords: acaricide resistance; ticks; monoterpenes; Brazilian pepper tree

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1. Introduction

Rhipicephalus microplus (Acari: Ixodidae) is the main ectoparasite of cattle in tropical and subtropical countries, causing significant economic losses to the beef and dairy industries. Infestations are associated with anemia, hide depreciation, delayed growth,

transmission of hemoparasites such as *Babesia* spp. and *Anaplasma* spp., and mortality, particularly in calves [1]. Annual economic losses associated with *R. microplus* infestations in Brazil have been estimated at approximately US$3.24 billion [2].

Conventional control of this tick relies mainly on the use of synthetic acaricides based on pyrethroids, organophosphates, macrocyclic lactones, and amidines [3]. These products are often marketed with limited regulatory oversight and are frequently used empirically by farmers, leading to inappropriate practices such as incorrect dilution rates, under- or overdosing, and prolonged use of the same molecule. In addition to increasing environmental impacts, these practices contribute substantially to the selection and development of resistance in tick populations to commercially available synthetic acaricides [3,4].

Plant-derived products have emerged as promising alternatives for the control of acaricide-resistant tick populations [5]. Essential oils (EOs) obtained from plant species exhibit a wide range of pharmacological properties, including acaricidal activity, and have been investigated as potential sources of bioactive compounds for tick control. Their toxicological profiles and potential effects on chemical residues may vary according to their chemical composition, concentration, formulation, and conditions of use. Therefore, under appropriate conditions, some plant-derived products may potentially contribute to reducing reliance on synthetic acaricides and, consequently, the occurrence of chemical residues in livestock production. However, these potential advantages require specific evaluation of toxicity, residues, and safety under the intended conditions of use [6–9].

Among these plants, *Schinus terebinthifolius* Raddi (Anacardiaceae), commonly known as Brazilian pepper tree, stands out due to its rich content of bioactive compounds, including terpenes and phenolic substances, which are associated with antimicrobial, anti-inflammatory, antiparasitic, and acaricidal activities [7,9,10]. Previous studies have demonstrated that the EOs of this species exhibit acaricidal, insecticidal, and repellent activities against arthropods, affecting physiological and reproductive performance [7,11]. However, despite strong evidence of its acaricidal potential, information comparing the efficacy of EOs obtained from different plant parts, such as leaves and fruits, remains limited.

Therefore, this study aimed to characterize the chemical composition of EOs obtained from leaves and fruits of *S. terebinthifolius*, to evaluate the in vitro acaricidal activity of EOs against *R. microplus*, and to assess its susceptibility to conventional acaricides.

2. Materials and Methods

2.1. Study Area

Engorged female *R. microplus* ticks were collected from naturally infested cattle on a dairy farm located in the municipality of Garanhuns (8°53′25″ S, 36°29′34″ W), Pernambuco State, Brazil. The study area has a mean annual temperature of 21 °C, ranging from 9 °C to 30 °C, and an average annual rainfall of approximately 700 mm [12–14].

Dairy farming is an important economic activity in the region, with a predominance of Holstein cattle [15]. Although highly productive, this breed is more susceptible to ectoparasite infestations, including *R. microplus* [1].

2.2. Botanical Material Collection, Extraction, and Characterization of Essential Oil

Leaves and fruits were collected from the same *S. terebinthifolius* tree in the municipality of Garanhuns, Pernambuco State, Brazil (8°54′24″ S, 36°29′40″ W), and kept separate for independent essential oil extraction. Collections were carried out between March and June 2025 from 6 to 9 am. A representative voucher specimen of *S. terebinthifolius*, properly identified, was deposited in the Dárdano de Andrade Lima Herbarium of the Agronomic Institute of Pernambuco (IPA) under registration number 94,315.

The plant material was dehydrated in a forced-air circulation oven at 50 °C until constant weight was achieved and subsequently ground using a knife mill. Essential oils were extracted by hydrodistillation using a 1:100 (*w*/*v*) ratio of plant material to distilled water for both plant parts (i.e., leaves and fruits). The procedure was performed using a Clevenger-type apparatus under light-protected conditions, with rapid heating to 100 °C followed by maintenance at a constant temperature for 2 h. The leaves and fruits EOs were obtained as single batches and used throughout the bioassays. Independent extraction batches were not prepared. At the end, the EO was separated from the hydrolate using a separatory funnel, transferred to amber glass vials, and stored at 3 °C until further analyses [16–19].

Furthermore, the yield was calculated based on the ratio between the mass of EO and the mass of plant material subjected to hydrodistillation [17]. The yields were 0.71% for the leaves and 6.25% for the fruits.

An aliquot of 1 µL of each EO was analyzed to determine its chemical composition using Gas Chromatography–Mass Spectrometry (GC–MS). Analyses were performed on a Shimadzu GCMS-QP2010 Ultra gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with an electron ionization source (70 eV) operating at 220 °C and fitted with an NST-01 capillary column (30 m × 0.25 mm × 0.25 µm). The oven temperature was programmed from 50 to 300 °C at a rate of 25 °C/min. A 1.0 µL sample was injected, with both the injector and detector maintained at 250 °C. Helium was used as the carrier gas at a flow rate of 1.2 mL/min.

The constituents were identified by comparing their mass spectra with those available in the NIST 11 spectral library and by comparing their experimental retention indices with literature values. Experimental retention indices were calculated using a homologous series of n-alkanes (C7–C40) analyzed under the same chromatographic conditions as the essential oil samples [20].

Methyl eugenol was used as an internal standard for the semiquantitative analysis of the essential oil constituents. The solution analyzed by GC-MS contained 0.1 mg of methyl eugenol and 0.2 mg of essential oil in 1 mL of dichloromethane. The relative abundance of each constituent was expressed as the percentage of the corresponding chromatographic peak area relative to the total integrated peak area. Because compound-specific calibration curves and response factors were not established, the reported values represent relative abundances rather than absolute concentrations. Accordingly, the chemical composition of the essential oils is presented as relative area (%) in Table 1.

Table 1. Chemical composition of essential oils obtained from the leaves and fruits of *Schinus terebinthifolius*, determined by gas chromatography-mass spectrometry. Relative area (%) represents the percentage of the total chromatographic peak area.

		EO of Leaves			EO of Fruits		
Compound	**KI Lit. (NST-01)**	**RT (min)**	**AR (%)**	**KI Exp.**	**RT (min)**	**AR (%)**	**KI Exp.**
1,7,7-trimethyltricyclo[2.2.1.02,6]heptane	924	8.393	0.15	925	-	-	-
α-Thujene	928	8.435	0.11	928	8.431	0.25	928
α-Pinene	937	8.542	21.15	937	8.541	37.13	936
Camphene	952	8.719	0.53	951	8.717	0.39	951
1-Isopropyl-4-methylenebicyclo[3.1.0]hex-2-ene	953	8.755	0.61	954	-	-	-
α-Sabinene	974	8.986	0.53	972	8.986	2.22	972
β-Pinene	978	9.054	7.41	977	9.054	13.81	978
β-Myrcene	985	9.148	0.42	985	9.147	8.88	985
α-Phellandrene	1001	-	-	-	9.337	3.42	1000

Table 1. *Cont.*

		EO of Leaves			EO of Fruits		
Compound	**KI Lit. (NST-01)**	**RT (min)**	**AR (%)**	**KI Exp.**	**RT (min)**	**AR (%)**	**KI Exp.**
3-Carene	1011	-	-	-	9.430	6.18	1010
o-Cymene	1017	9.497	0.62	1017	9.500	0.32	1017
β-Phellandrene	1027	-	-	-	9.602	5.31	1027
Isosylvestrene	1028	9.603	1.07	1028	-	-	-
p-Cymenene	1078	10.105	0.19	1079	-	-	-
α-Terpinolene	1080	-	-	-	10.166	0.26	1086
Linalool	1086	10.173	0.25	1086	-	-	-
α-Campholenal	1114	10.416	0.76	1114	-	-	-
trans-Pinocarveol	1139	10.610	2.91	1138	-	-	-
Bicyclo[3.2.0]heptan-3-ol, 2-methylene-6,6-dimethyl	1148	10.690	0.24	1148	-	-	-
p-Mentha-1,5-dien-8-ol	1155	10.758	1.27	1156	-	-	-
p-Cymen-8-ol	1171	10.863	0.67	1169	-	-	-
Terpinen-4-ol	1176	10.905	0.55	1175	10.905	0.34	1175
(1R)-(-)-Myrtenal	1188	10.991	1.75	1185	-	-	-
Myrtenol	1190	11.043	1.14	1192	-	-	-
4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one	1196	11.083	0.75	1197	-	-	-
p-Mentha-6,8-dien-2-ol	1207	11.164	0.26	1207	-	-	-
(Z)-3,7-dimethyl-2,6-Octadienal	1222	11.270	1.18	1223	-	-	-
(E)-3,7-dimethyl-2,6-Octadienal	1252	11.461	1.44	1250	-	-	-
Carvacrol	1280	11.679	1.08	1280	-	-	-
Bornyl acetate	1282	-	-	-	11.702	0.39	1284
α-Cubebene	1360	12.248	0.26	1368	-	-	-
Copaene	1387	12.454	5.10	1401	12.453	0.63	1400
β-Elemene	1402	12.500	1.87	1408	-	-	-
Isopulegyl acetate	1435	12.655	0.17	1435	-	-	-
Isocaryophyllene	1451	12.752	2.08	1451	12.753	0.89	1451
Germacrene A	1482	-	-	-	12.945	0.26	1484
α-Caryophyllene	1484	12.949	0.44	1484	-	-	-
Aromandendrene	1495	13.004	0.90	1494	-	-	-
Isogermacrene D	1508	13.087	3.75	1508	-	-	-
Caryophyllene	1518	13.139	5.27	1518	-	-	-
δ-Cadinene	1530	13.246	6.43	1537	13.085	9.32	1528
10-epi-Elemol	1540	-	-	-	13.251	0.91	1538
Ylangene	1546	13.295	1.07	1546	-	-	-
9-Methoxycalamenene	1559	13.368	2.32	1559	-	-	-
γ-Eudesmol	1561	-	-	-	13.379	4.37	1561
Spatulenol	1603	13.607	2.46	1603	-	-	-
Caryophyllene oxide	1614	13.669	1.57	1614	-	-	-
Epiglobulol	1626	13.727	1.90	1626	-	-	-
Cubenol	1640	13.798	1.48	1639	-	-	-
β-Eudesmol	1642	-	-	-	13.884	1.87	1646
τ-Cadinol	1644	13.900	5.59	1659	-	-	-
α-Cadinol	1655	13.978	1.67	1674	-	-	-
Hedycaryol	1680	14.010	1.96	1680	-	-	-

Table 1. *Cont.*

Compound	KI Lit. (NST-01)	EO of Leaves			EO of Fruits		
		RT (min)	AR (%)	KI Exp.	RT (min)	AR (%)	KI Exp.
1,1,4a-Trimethyl-5,6-dimethylenedecahydronaphthalene	1690	14.091	0.85	1696	-	-	-
Androstan-17-one, 3-ethyl-3-hydroxy-, (5α)	1745	14.333	0.60	1745	-	-	-
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	1790	14.555	0.43	1790	-	-	-
3-Isopropyl-6,7-dimethyltricyclo[4.4.0.0(2,8)]decane-9,10-diol	1797	14.591	0.38	1797	-	-	-
Total identified (%)			95.59			97.15	

KI Lit. (NST-01): retention index reported in the literature for the NST-01 capillary column; KI Exp.: experimentally determined retention index; RT: retention time (min); AR (%): relative peak area (%); EO: essential oil.

2.3. Collection of Engorged Females, Identification, and Acaricidal Assays

Engorged female *R. microplus* were manually collected from naturally infested cattle that had not received any ectoparasiticide treatment for at least 30 days prior to sampling. The specimens were stored in plastic vials and transported to the laboratory, where they were morphologically identified under a stereomicroscope [3,21], counted, and distributed into experimental groups. The collected engorged females showed similar body sizes and were distributed among the experimental groups to obtain comparable total weights, with a maximum difference of 0.2 g between Petri dishes.

All assays were performed in triplicate. Engorged females were distributed into 15 homogeneous groups, each consisting of 10 specimens per Petri dish (approximately 1.6 g per dish), totaling 45 dishes. Each group was considered an independent experimental unit and statistical replicate. The in vitro acaricidal activity of the EOs obtained from the leaves and fruits of *S. terebinthifolius* was evaluated at concentrations of 1.0%, 0.5%, and 0.25%. Two negative control groups were included: distilled water and 6%Tween 80.

In addition, seven commercial synthetic acaricide formulations commonly used by cattle producers in the study area were evaluated. These formulations were selected based on their use by local cattle producers rather than on previously reported efficacy [4]. The active ingredient compositions of the evaluated formulations were: (I) cypermethrin 15.0 g + chlorpyrifos 25.0 g + piperonyl butoxide 10.0 g/100 mL; (II) amitraz 12.5 g/100 mL; (III) dichlorvos 60.0 g + chlorfenvinphos 16.65 g + calcium alkylbenzene sulfonate 20.0 g + xylene 73.1 g/100 mL; (IV) deltamethrin 50.0 g/100 mL; (V) alpha-cypermethrin 5.0 g + ethion 16.0 g + chlorpyrifos 8.5 g/100 mL; (VI) cypermethrin 20.0 g + chlorpyrifos 50.0 g/100 mL; and (VII) cypermethrin 15.0 g + chlorpyrifos 25.0 g + citronellal 1.0 g/100 mL. Each commercial formulation was diluted in distilled water according to the manufacturer-recommended dilution prior to the Adult Immersion Test (AIT). The recommended dilutions were: (I) 1.25 mL/L of water; (II) 2.0 mL/L of water; (III) 2.5 mL/L of water; (IV) 1.0 mL/L of water; (V) 2.5 mL/L of water; (VI) 0.85 mL/L of water; and (VII) 1.0 mL/L of water. These concentrations were selected for the experimental evaluation of the EOs and were not intended to provide dose-equivalent comparisons with the commercial acaricide formulations.

The efficacy of each treatment was assessed using the AIT [22]. Briefly, EOs were prepared at concentrations of 1.0% (*v*/*v*), 0.5% (*v*/*v*), and 0.25% (*v*/*v*), corresponding to 1.0, 0.5, and 0.25 mL of essential oil per 100 mL of test solution, respectively. Tween 80 was used at a final concentration of 6% as an emulsifying agent to facilitate the dispersion of the EOs in distilled water [17]. Each experimental group was immersed in its respective treatment solution for 5 min [4,17,22]. Subsequently, the engorged females were dried out

on filter paper and maintained in an incubator under controlled conditions (26 ± 1 °C and 80 ± 5% relative humidity) for 15 days to allow oviposition. The egg masses were weighed, transferred to hermetically sealed tubes, and incubated under the same controlled conditions. Egg hatchability was evaluated after 30 days of incubation by separating and counting the hatched larvae under a stereomicroscope. The egg hatching rate was expressed as the percentage of larvae relative to the total number of eggs incubated. Mortality of engorged females was recorded daily throughout the oviposition period.

2.4. Statistical Analysis

Data were initially analyzed through descriptive analysis to determine absolute and relative frequencies. Treatment efficacy was calculated based on the mean values obtained from the three independent Petri-dish replicates for each treatment. The Petri dish was considered the experimental and statistical unit, and individual ticks were not treated as independent observations.

The reproductive efficiency (RE) of engorged females was estimated through a mathematical relation among egg mass weight (g), egg hatchability (%), and engorged female weight (g), which allowed a standardized assessment of the reproductive performance of ticks exposed to the different compounds. Oviposition reduction (OR) was calculated using a proportional method based on the comparison of egg masses between the control and treated groups. Treatment efficacy (TE) was determined by comparing the reproductive efficiency of the treated groups with that of the control group. The formulas for reproductive efficiency, treatment efficacy and oviposition reduction are presented below. The value of 20,000 represents the estimated number of larvae per gram of eggs [22].

$$\text{Reproductive efficiency(RE)} = \frac{\text{egg mass(g)} \times \text{egg hatchability(\%)} \times 20,000}{\text{engorged female weight(g)}}$$

$$\text{Treatment efficacy(TE)} = \frac{\text{RE of the control group} - \text{RE of the treated group}}{\text{RE of the control group}} \times 100$$

$$\text{Oviposition reduction(\%)} = \frac{\text{egg mass in the control group(g)} - \text{egg mass in the treated group(g)}}{\text{egg mass in the control group(g)}} \times 100$$

Results were expressed as mean ± standard error (SE), calculated from the experimental replicates. Distilled water was used as the reference negative control for the calculation of RE, TE and related parameters. A 6% Tween 80 solution was included as an additional control to verify that the emulsifying agent itself did not exhibit acaricidal activity [17]. Acaricidal efficacy (AE) was calculated based on engorged female weight, egg mass weight, and egg hatchability [22]. Treatments showing efficacy equal to or greater than 95% were considered effective for the control of *R. microplus* [4,17,22].

The relationship between EO concentration and tick reproductive parameters was explored descriptively using Pearson's correlation coefficient (r), based on the mean values obtained from the three independent experimental replicates at each concentration. Thus, the correlation analysis comprised three concentration levels (0.25%, 0.5%, and 1.0%; n = 3). The coefficient of determination (r^2) was calculated to describe the proportion of variation in the mean reproductive parameters associated with the linear relationship with EO concentration. Statistical procedures were performed using BioEstat version 5.3 (Belém, Brazil) and MedCalc Statistical Software Ltd. version 6.1 (Ostend, Belgium) for Windows.

3. Results

The chemical composition of the EOs extracted from the leaves and fruits of *S. terebinthifolius* is presented in Table 1.

A total of 48 compounds were identified in the EO of *S. terebinthifolius* from leaves, representing 95.59% of the total chromatographic peak area. A predominance of monoter-

penes and sesquiterpenes was detected. The major constituents were α-pinene (21.15%), β-pinene (7.41%), δ-cadinene (6.43%), τ-cadinol (5.59%), and copaene (5.10%). In the EO from fruit, compounds accounted for 97.15% of the total chromatographic peak area and exhibited a chemical profile distinct from that of the leaves. A total of 20 compounds were identified, predominantly monoterpenes, with α-pinene (37.13%), β-pinene (13.81%), β-myrcene (8.88%), δ-cadinene (9.32%), and 3-carene (6.18%) being the major constituents. Overall, the EO from fruit showed a higher proportion of major compounds in terms of relative area than the leaf EO, despite its lower chemical diversity.

The effects of the different treatments on engorged female *R. microplus* ticks and their reproductive parameters are presented in Table 2.

Table 2. Effect of commercial acaricides and essential oils from the leaves and fruits of *Schinus terebinthifolius* on mortality, oviposition reduction, hatchability rate, reproductive efficiency, and treatment efficacy in engorged female *Rhipicephalus microplus* ticks.

Treatment	Engorged Female Mortality (%)	Oviposition Reduction (%)	Hatchability Rate (%)	Reproductive Efficiency (%)	Treatment Efficacy (%)
Distilled water	0 ± 0.0	NA	100.0 ± 0.0	100.0 ± 0.0	NA
Tween 80	0 ± 0.0	NA	100.0 ± 0.0	100.0 ± 0.0	NA
EO (L) 1%	16.67 ± 3.33	72.37 ± 2.28	12.0 ± 2.08	3.31 ± 0.39	96.69 ± 0.39
EO (L) 0.5%	0 ± 0.0	40.79 ± 4.25	32.0 ± 2.31	18.95 ± 1.58	81.05 ± 1.58
EO (L) 0.25%	0 ± 0.0	30.26 ± 3.54	83.0 ± 3.61	57.88 ± 1.47	42.12 ± 1.47
EO (F) 1%	20.0 ± 2.89	73.68 ± 4.00	8.0 ± 1.73	2.1 ± 0.94	97.9 ± 0.94
EO (F) 0.5%	6.67 ± 3.33	44.74 ± 2.28	29.0 ± 1.00	16.02 ± 0.84	83.98 ± 0.84
EO (F) 0.25%	0 ± 0.0	27.63 ± 4.27	80.0 ± 5.03	59.97 ± 1.81	40.03 ± 1.81
I	40.0 ± 5.77	76.32 ± 2.38	15.0 ± 2.89	3.55 ± 2.20	96.45 ± 2.20
II	0 ± 0.0	18.42 ± 4.56	55.0 ± 2.65	44.86 ± 4.61	55.14 ± 4.61
III	100.0 ± 0.0	NA	NA	0 ± 0.0	100.0 ± 0.0
IV	0 ± 0.0	1.32 ± 0.76	87.5 ± 1.45	86.35 ± 1.93	13.65 ± 1.93
V	10.0 ± 0.0	38.16 ± 4.56	25.0 ± 1.73	15.46 ± 2.21	84.54 ± 2.21
VI	3.33 ± 3.33	15.79 ± 4.94	80.0 ± 2.65	67.36 ± 6.15	32.64 ± 6.15
VII	6.67 ± 3.33	43.42 ± 2.70	82.5 ± 2.96	46.67 ± 1.39	53.33 ± 1.39

NA = Not applicable; EO (L) = Essential oil from the leaves of *Schinus terebinthifolius*; EO (F) = Essential oil from the fruits of *S. terebinthifolius*; I = cypermethrin 15.0 g + chlorpyrifos 25.0 g + piperonyl butoxide 10.0 g/100 mL; II = amitraz 12.5 g/100 mL; III = dichlorvos 60.0 g + chlorfenvinphos 16.65 g + calcium alkylbenzene sulfonate 20.0 g + xylene 73.1 g/100 mL; IV = deltamethrin 50.0 g/100 mL; V = alpha-cypermethrin 5.0 g + ethion 16.0 g + chlorpyrifos 8.5 g/100 mL; VI = cypermethrin 20.0 g + chlorpyrifos 50.0 g/100 mL; and VII = cypermethrin 15.0 g + chlorpyrifos 25.0 g + citronellal 1.0 g/100 mL. Values are expressed as mean ± standard error % (SE).

The EOs of *S. terebinthifolius* exhibited concentration-dependent acaricidal activity. At a concentration of 1%, the leaf and fruit EOs achieved efficacies of 96.69% and 97.90%, respectively. At these concentrations, mortality rates of engorged female ticks were 16.67% for the leaf EO and 20.0% for the fruit EO. Reducing the concentration to 0.5% and 0.25% resulted in a progressive decline in treatment efficacy, accompanied by increased reproductive efficiency and egg hatchability.

Among the commercial acaricides evaluated, only treatments I (cypermethrin + chlorpyrifos + piperonyl butoxide) and III (dichlorvos + chlorfenvinphos + calcium alkylbenzene sulfonate + xylene) achieved efficacy values above 95%, reaching 96.45% and 100%, respectively. Treatment III caused complete mortality of engorged females, whereas treatment I resulted in 40% mortality. The remaining products showed efficacy values below the threshold recommended for the control of *R. microplus*, ranging from 13.65% to 84.54%. These values describe the performance of the commercial acaricide formulations under the specific conditions of the AIT and should not be interpreted as directly comparable in potency with the essential oils, which were evaluated at experimentally defined concentrations.

A positive correlation was observed between leaf EO concentration and oviposition inhibition by engorged females ($r = 0.8386$; $r^2 = 0.70$). Negative correlations were observed between EO concentration and larval hatchability ($r = -0.4500$; $r^2 = 0.20$) and between EO concentration and reproductive efficiency ($r = -0.4548$; $r^2 = 0.21$). At the highest concentration tested (1%), reproductive efficiency was reduced to 3.31%.

Similar patterns were observed for the fruit EO. A positive correlation was observed between EO concentration and oviposition inhibition ($r = 0.7585$; $r^2 = 0.58$), whereas negative correlations were observed with larval hatchability ($r = -0.4597$; $r^2 = 0.21$) and reproductive efficiency ($r = -0.4102$; $r^2 = 0.17$). At the highest concentration evaluated, reproductive efficiency was reduced to 2.10%.

Although direct mortality of engorged females was low in most EO treatments, reductions in oviposition, egg hatchability, and reproductive efficiency were observed, particularly at the 1% concentration. Likewise, treatments I and V reduced reproductive parameters despite causing relatively low mortality of engorged females.

4. Discussion

This study provides evidence of the in vitro acaricidal activity of 1% EOs of leaves and fruits of *S. terebinthifolius*, demonstrating promising potential as an alternative for the control of *R. microplus*. Furthermore, the relatively limited efficacy observed at lower concentrations may be related to the reduced availability of bioactive constituents, while the higher efficacy at 1% suggests a concentration-dependent effect [7].

The differences observed in the chemical composition of the EOs extracted from the leaves and fruits of *S. terebinthifolius* revealed important variations in both the diversity and distribution of their constituents. The EO from leaves exhibited greater chemical complexity, with a higher number of compounds distributed at lower relative abundances. In contrast, the EO from fruit showed lower chemical diversity but a higher concentration of major constituents, particularly monoterpenes such as α-pinene and β-pinene. However, a limitation of the present study is that the actual amount of essential oil to which each tick was exposed was not determined, as the assays were based on nominal concentrations. However, the experimental conditions were standardized among replicates, including the number and weight of specimens and the concentration and volume of the test solutions.

Differences in chemical composition directly influence the biological properties of the EOs, supporting a comparative analysis of the different plant parts. In general, the leaves of aromatic plants tend to exhibit greater diversity of bioactive compounds than fruits, which may be associated with the higher abundance of specialized secretory structures, such as trichomes, responsible for the synthesis and storage of secondary metabolites [10,23]. In *S. terebinthifolius*, this characteristic contributes to the formation of more complex chemical mixtures in the leaf EO, which may result in distinct mechanisms of action and biological activities compared with the fruit EO, according to their respective chemical compositions. These differences may also be associated with geographic origin, climatic and seasonal conditions, phenological stage, and extraction procedures, which can influence the relative abundance of secondary metabolites in essential oils [10,23,24].

Variations in the chemical composition of EOs among different parts of the same plant species reflect differences in both the diversity and predominance of their constituents. In another study evaluating *S. terebinthifolius*, 22 compounds were identified in the leaf EO and 13 in the fruit EO, with caryophyllene (15.97%) and germacrene D (12.04%) predominating in the leaves, whereas β-pinene (43.34%) and α-pinene (17.16%) were the major constituents in the fruits [10]. These findings reinforce that the distribution of secondary metabolites is not uniform throughout the plant and may vary according to the plant material analyzed.

These chemical differences may be directly associated with the effects observed on the biological parameters of *R. microplus*. The statistical analyses demonstrated that the acaricidal activity of *S. terebinthifolius* EOs was concentration-dependent. The positive correlation between EO concentration and oviposition inhibition indicates that increasing concentrations progressively impair the reproductive capacity of engorged females. Likewise, the negative correlation observed for egg hatchability and reproductive efficiency demonstrates that higher concentrations progressively reduce the reproductive performance of ticks, affecting not only the survival of exposed individuals but also the offspring.

The efficacy of the leaf and fruit EOs of *S. terebinthifolius* at a concentration of 1% against engorged female *R. microplus* observed in the present study may be associated with the presence of monoterpenes and sesquiterpenes, including α-pinene, β-pinene, β-myrcene, δ-cadinene, copaene, and 3-carene. These compounds, present in both EOs, are lipophilic and may facilitate their interaction with the tick cuticle and cellular membranes [5,7,25–27].

Previous studies have proposed that some constituents of EOs may exert acaricidal effects through multiple mechanisms, including interference with neuronal function and inhibition of acetylcholinesterase (AChE) in specific experimental systems [25–27]. Inhibition of AChE can result in the accumulation of acetylcholine at synaptic junctions, leading to neuronal hyperstimulation, impaired motor coordination, paralysis, and ultimately death. However, these mechanisms were not experimentally evaluated in the present study and should therefore be considered possible mechanisms underlying the observed acaricidal activity, which may reflect the combined effects of multiple constituents and potential synergistic, additive, or antagonistic interactions that were not evaluated herein.

In a study evaluating the EO obtained from fresh leaves, marked activity against both adult and larval stages of *R. microplus* was observed. Rich in terpenes, the EO induced high mortality of engorged females and exhibited significant larvicidal activity. The authors attributed these effects mainly to the action of bioactive compounds on the tick nervous system, particularly through AChE inhibition, with biological activity detected even at low concentrations, such as 0.0003 mg/mL [7].

The results observed against *R. microplus* do not represent an occasional finding. The biological activity of *S. terebinthifolius* EOs has also been reported against other arthropods of economic importance. In this context, the leaf EO, containing 32 compounds with limonene (23.22%) as the major constituent, and the fruit EO, containing 27 compounds with γ-elemene (10.11%) as the predominant compound, exhibited insecticidal and repellent activities against *Sitophilus oryzae* (rice weevil) [28]. These findings further support the potential of *S. terebinthifolius* EOs as an alternative tool for arthropod management, particularly in scenarios where resistance to conventional chemical compounds is established.

In the present study, among the formulations containing pyrethroids, only treatment I (cypermethrin 15.0 g + chlorpyrifos 25.0 g + piperonyl butoxide 10.0 g) achieved the efficacy threshold (≥95%) established [22]. Nevertheless, this formulation caused only partial mortality of engorged females and exerted its primary effects on reproductive parameters, reducing oviposition and larval hatchability. The remaining pyrethroid-based formulations, whether used alone or in combination with other active ingredients, exhibited low efficacy, highlighting the reduced susceptibility to acaricides of the evaluated tick population. Reduced susceptibility to acaricides has been associated with mechanisms such as mutations in voltage-gated sodium channels, which may reduce pyrethroid efficacy and contribute to reduced susceptibility to compounds such as cypermethrin and deltamethrin [29–31].

Previous studies have reported higher susceptibility of *R. microplus* populations to similar formulations, particularly the combination of cypermethrin and chlorpyrifos, which achieved an average efficacy of approximately 80% using ticks collected in the same studied

area [31]. However, the findings of the present study indicate a different trend, characterized by reduced efficacy of most of these formulations, which may reflect differences in acaricide susceptibility among *R. microplus* populations.

This situation was particularly evident for deltamethrin. This compound exhibited poor performance against engorged females and did not produce substantial changes in reproductive parameters, which remained close to those observed in the control group. Wide variation in the efficacy of deltamethrin has been reported [32]. This heterogeneous response pattern may reflect differences in susceptibility among tick populations. However, the present study evaluated a single tick population, and the mechanisms underlying the observed response were not investigated.

Amitraz also failed to reach the efficacy threshold. In regions where its use remains limited, this formamidine compound exhibits high acaricidal activity due to its action on octopamine receptors, which regulate behavior, locomotion, and feeding in ticks [33]. In Brazil, variations in the response of tick populations to amitraz have been reported, with reduced efficacy frequently associated with its continuous use, inappropriate application practices, and the absence of management strategies capable of minimizing the selection of resistant individuals [32,34–36].

The organophosphate-containing formulations showed variable efficacy against the *R. microplus* population evaluated in this study. High efficacy was observed in formulations I and III, which achieved 96.45% and 100% efficacy, respectively. Formulation I contained piperonyl butoxide, a synergistic agent that may enhance the activity of certain insecticides, whereas formulation III achieved 100% efficacy without this compound. In contrast, formulation VI, which contained higher concentrations of cypermethrin and chlorpyrifos than formulation I but lacked piperonyl butoxide, showed low efficacy (32.64%). Organophosphates act through the inhibition of acetylcholinesterase (AChE), a mechanism associated with their neurotoxic effects on ticks [22,30,37].

In treatment VII (cypermethrin 15 g + chlorpyrifos 25 g + citronellal 1.0 g), the inclusion of citronellal did not result in high acaricidal efficacy. Citronellal has been reported to exhibit acaricidal and insecticidal activity in previous studies, although its effects may vary according to the target arthropod, concentration, and experimental conditions [25,38–40]. However, the observed activity reflects the combined formulation, and the specific contribution of citronellal cannot be established from the present results.

This study has some limitations that should be considered when interpreting the findings. The evaluation was conducted using a single *R. microplus* population from one farm, which limits the generalizability of the results to other tick populations. Only three EO concentrations and three experimental replicates were evaluated, restricting the assessment of concentration-dependent effects. Finally, in vivo studies are needed to evaluate the efficacy, safety, and practical applicability of the EOs under field conditions.

Therefore, the reduced susceptibility to most synthetic acaricides observed in the present study highlights the need for effective alternatives for the control of *R. microplus*. In this context, the leaf and fruit EOs of *S. terebinthifolius* exhibited acaricidal effects, particularly on the reproductive parameters of *R. microplus* teleogines, which may be associated with the presence of bioactive compounds [7,41]. The observed alterations in reproductive performance may impair the reproductive success of engorged females and, consequently, contribute to reducing the population growth of the tick. These findings support the potential of *S. terebinthifolius* EOs as alternatives for tick control, although further studies are needed to elucidate their specific mechanisms of action.

5. Conclusions

The leaves and fruit EOs of *S. terebinthifolius* demonstrated acaricidal activity against engorged females of *R. microplus* under in vitro conditions, with the most pronounced effects observed on reproductive parameters, including oviposition, egg hatchability, and reproductive efficiency. The *R. microplus* population evaluated in this study also showed reduced susceptibility to most of the synthetic acaricide formulations tested, resulting in limited efficacy of several commonly used products. These findings indicate that the essential oils of *S. terebinthifolius* may warrant further investigation as potential candidates for tick control. Further studies, including mechanistic, toxicity, and in vivo evaluations, are needed to determine their efficacy, safety, and practical applicability under field conditions.

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