Anthelmintic efficacy of *Lippia alba* essential oil against gastrointestinal nematodes in goats

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Abstract
This study aimed to evaluate the anthelmintic efficacy of *Lippia alba* essential oil against gastrointestinal nematodes in goats, identify the helminth genera present, and perform a chemical characterization of the tested oil. Fecal samples were collected and quantified using the McMaster technique; samples with counts above 200 eggs per gram (EPG) were included in the experiment. The pooled positive sample showed an EPG of 2800. Quantitative coproculture was conducted with a negative control group (distilled water) and *L. alba* essential oil at concentrations of 10 mg/mL, 25 mg/mL, 50 mg/mL, and 100 mg/mL, maintained at room temperature for 10 days. Subsequently, larvae were recovered, identified, and quantified. Treatment efficacy was assessed using the larval reduction formula. Concentrations of 25 mg/mL, 50 mg/mL, and 100 mg/mL were effective, achieving larval reductions of 79.5%, 87.8%, and 89.8%, respectively. The genera identified included *Haemonchus* sp. (56.8%), *Trichostrongylus* sp. (42.9%), and *Oesophagostomum* sp. (0.3%). Chemical composition of the oil was analyzed using gas chromatography-mass spectrometry with an advanced electron ionization source. Twenty-five compounds were identified, with E-citral (23.76%), Z-citral (20.46%), carvone (10.92%), and limonene (6.40%) being the most abundant. All tested concentrations of *L. alba* essential oil, except 10 mg/mL (29.7%), demonstrated anthelmintic potential by significantly reducing gastrointestinal nematode larvae in goats.
Keywords: botanical compounds; helminthiasis; *Lippia alba*; parasitic resistance; Verbenaceae.

Eficácia anti-helmíntica do óleo essencial de Lippia alba contra nematoides gastrintestinais em caprinos

Resumo
Este estudo teve como objetivo avaliar a eficácia anti-helmíntica do óleo essencial de Lippia alba contra nematoides gastrointestinais em caprinos, identificar os gêneros de helmintos presentes e realizar a caracterização química do óleo testado. Amostras fecais foram coletadas e quantificadas pela técnica de McMaster; amostras com contagens acima de 200 ovos por grama (OPG) foram incluídas no experimento. A amostra positiva combinada apresentou um OPG de 2.800. A coprocultura quantitativa foi conduzida com um grupo controle negativo (água destilada) e óleo essencial de L. alba nas concentrações de 10 mg/mL, 25 mg/mL, 50 mg/mL e 100 mg/mL, mantidos em temperatura ambiente por 10 dias. Posteriormente, as larvas foram recuperadas, identificadas e quantificadas. A eficácia do tratamento foi avaliada pela fórmula de redução larval. As concentrações de 25 mg/mL, 50 mg/mL e 100 mg/mL foram eficazes, alcançando reduções larvais de 79,5%, 87,8% e 89,8%, respectivamente. Os gêneros identificados incluíram Haemonchus sp. (56,8%), Trichostrongylus sp. (42,9%) e Oesophagostomum sp. (0,3%). A composição química do óleo foi analisada por cromatografia gasosa acoplada a espectrometria de massas com uma fonte avançada de ionização de elétrons. Vinte e cinco compostos foram identificados, sendo E-citral (23,76%), Z-citral (20,46%), carvona (10,92%) e limoneno (6,40%) os mais abundantes. Todas as concentrações testadas de óleo essencial de L. alba, exceto 10 mg/mL (29,7%), demonstraram potencial anti-helmíntico, reduzindo significativamente as larvas de nematoides gastrointestinais em cabras.

Palavras-chave: *compostos botânicos; helmintoses; Lippia alba; resistência parasitária; Verbenaceae.*

1 Introduction

Goat farming is widely practiced in tropical regions, focusing on the sustainable production of meat, milk, and hides (Dias *et al*., 2022). It is a vital source of animal protein and economic income, playing an important socioeconomic role, especially for rural communities (Chniter *et al*., 2024). In recent years, interest in goat farming has grown in developed countries as well, driven by technological advances that improve productivity (Dias *et al*., 2022).

It is well established that goats are more susceptible to nematodes than sheep and cattle, a condition attributed to their physiological characteristics, thereby necessitating greater attention to sanitary management (Fthenakis; Papadopoulos, 2018). One of the primary health challenges in goat production is infestation by gastrointestinal nematodes (GINs), which cause substantial economic losses due to reduced productivity, manifested as inappetence, anemia, diarrhea, stunted growth, and, in severe cases, death (León; Delgado; Florez, 2019). In Brazil, helminthiasis ranks among the most serious health problems affecting goat herds, particularly in extensive production systems where climatic conditions favor the survival and dissemination of infective larvae. The most frequently reported etiological agents in Northeastern Brazil include *Haemonchus spp*., the most epidemiologically significant, followed by *Strongyloides spp*. (Oliveira et al., 2018), *Trichostrongylus spp*. (Salgado; Santos, 2016), and *Oesophagostomum spp*. (Maia; Mattos, 2020). Moreover, the widespread use of anthelmintics has contributed to increased drug resistance and recurrence of infections (Macedo et al., 2023).

Easy access to commercial anthelmintics has resulted in their improper use, accelerating the development of resistance to the main available drugs (Silva et al., 2018). This scenario hinders parasite control and heightens the economic burden on producers. Consequently, the implementation of integrated parasite management strategies is urgently required, combining the rational use of anthelmintics with alternative control measures. Such strategies include proper dosing, annual rotation of drug classes, pasture management, and routine monitoring of fecal egg counts (Esteban-Ballesteros et al., 2017).

Beyond the issue of resistance, the use of endectocides raises environmental concerns, as approximately 98% of the administered compounds are excreted unchanged in feces. This may adversely affect non-target soil organisms and disrupt ecosystem balance (Soares *et al*., 2023; Pavlović *et al*., 2019; Snow *et al.,* 2019).

Given these challenges, the use of phytotherapeutic agents to control helminthiasis is gaining interest. These alternatives are generally non-toxic to treated animals, do not leave residues in meat or milk, and are cost-effective (Dias *et al*., 2022). In this context, essential oils (EOs) with anthelmintic properties have emerged as a promising strategy and are officially recognized in Brazil through Normative Instruction Nº 46, issued by the Ministry of Agriculture, Livestock and Supply (Brazil, 2011).

The Verbenaceae family is prominent within this framework, with about 100 genera and 3,000 species, widely distributed across tropical, subtropical, and temperate zones in the Americas, Africa, and Asia. Several species in this family are known for their bioactive potential, especially *Lippia alba*. Commonly known as lemon balm, *L. alba* is a shrub rich in essential oil, mainly containing citral. Its EO has shown bactericidal, fungicidal (Oliveira *et al*., 2014; Olivero-Verbel *et al*., 2014), acaricidal, insecticidal (Vera *et al*., 2014), and trypanocidal (Baldissera *et al*., 2017) activities. More recently, its anthelmintic potential has been investigated, revealing promising ovicidal and adulticidal effects, especially against *Haemonchus contortus* (Barbosa *et al*., 2023). These effects may involve disrupting the lipid layers of helminth eggs, increasing membrane permeability, and interfering with larval development or adult parasite survival (André *et al*., 2018; Cao *et al*., 2021).

The selection of *L. alba* (Verbenaceae) for this study was based on existing literature identifying it as a plant with high essential oil yield and bioactivity. Yet, it is remains underexplored in controlling gastrointestinal nematodes in goats. This gap highlights the need for further research into its potential as a sustainable anthelmintic alternative.

The remainder of this article describes, in Section 2, the materials and methods employed for

the extraction and chemical characterization of Lippia alba essential oil, as well as the procedures used in in vitro biological assays to evaluate its anthelmintic activity against gastrointestinal nematodes in goats. Section 3 presents the results along with a discussion comparing the findings with previous studies. Finally, Section 4 provides the conclusions and recommendations for future research on the potential use of this essential oil as an alternative method for helminth control.

2 Material and methods

2.1 Ethical compliance and plant material handling

This study was conducted following approval by the Animal Use Ethics Committee (AUEC) of the Federal University of Recôncavo da Bahia (UFRB), under protocol number 23007.00016885/2022-67.

Specimens of *Lippia alba* were collected from shrubs located on the Experimental Farm and surrounding laboratory blocks at UFRB in Cruz das Almas, Bahia, Brazil, in September 2023. The collection site coordinates are 12°40'39" S and 39°06'26" W, at an elevation of 226 m. A voucher specimen was deposited at the UFRB Herbarium (HURB), under registration number HURB 8794. The aerial parts of the plant were used for essential oil extraction. After collection, the plant material was stored in brown paper bags and dried in a laboratory oven (Block L, UFRB) at 44°C for 3 to 5 days.

The essential oil was extracted from dehydrated leaves by hydrodistillation using a Clevenger-type apparatus. In 2000 mL and 1000 mL volumetric flasks, 60 g and 30 g of dried leaves were added, respectively, with sufficient distilled water to immerse the plant material (approximately 1000 mL and 500 mL). During extraction, ice packs were placed in a thermostatic bath to maintain a temperature of 4°C. The extraction process lasted 2 hours, starting when internal water flow through the condenser commenced. The collected oil was retrieved with a Pasteur pipette and stored in amber vials (5 mL) under refrigeration until use.

For in vitro assays, the essential oil was tested at concentrations of 10, 25, 50, and 100 mg/mL to assess its inhibitory effect on larval development (egg to L3 stage). These concentrations were defined based on previous studies reporting significant anthelmintic activity of essential oils. The appropriate quantities of essential oil were weighed using an analytical balance and diluted in 2 mL of distilled water containing 3% Tween 80 to ensure uniform dispersion in the aqueous medium.

2.2 Fecal sample collection and biological assay

Fecal samples were obtained from 27 goats housed in the Goat and Sheep Sector of the Experimental Farm at UFRB, which had not received synthetic anthelmintics for at least 30 days. Collection was performed directly from the rectum and analyzed at the Laboratory of Parasitology and Parasitic Diseases (LPDP) of the University Veterinary Hospital (HUMV/UFRB). Samples were screened via the eggs-per-gram (EPG) method, and only 18 samples presenting EPG counts greater than 200, predominantly from Trichostrongyloidea and Chabertidae families, were included in the assays.

The coproculture assay was conducted using the methodology described by Ueno and Gonçalves (1998), adapted for this study. A pooled sample was created from positive feces, reaching a count of 2800 EPG. Treatment groups were prepared by mixing 2 g of pooled feces, 2 g of sawdust, and 2 mL of essential oil solution (at the respective concentrations with 3% Tween 80 and distilled water). The negative control group consisted of 2 g of feces, 2 g of sawdust, and 2 mL of the emulsifier solution without essential oil. Each treatment was replicated 10 times.

The coprocultures were sealed with perforated PVC film for aeration and incubated at room temperature for 10 days. After incubation, the cultures were filled with distilled water heated to 40 °C, covered with Petri dishes, and inverted to facilitate larval migration. After 2 hours, 10 mL of distilled water was added along the inner walls of each container. The resulting suspension was collected using a Pasteur pipette and transferred to labeled 15 mL Falcon tubes for refrigeration.

Samples were centrifuged at 2000 rpm for 5 minutes. The supernatant was discarded, retaining 2 mL of concentrate. Larvae were counted using a Sedgwick Rafter counting chamber (S50) stained with Lugol and examined under a light microscope.

Data were analyzed via analysis of variance (ANOVA), followed by Tukey's post hoc test at a 5% significance level. The anthelmintic efficacy of the essential oil was calculated using the larval reduction formula from Rodrigues *et al*. (1996):

$$\%reduction = \frac{number\ of\ control\ larvae - number\ of\ \ treated\ larvae}{number\ of\ control\ larvae} \times 100 \qquad (1)$$

2.3 Chemical characterization of the essential oil of *Lippia alba*

An aliquot of the essential oil was submitted to the Regional Center for Technological Development and Innovation (CRTI – Goiás, Brazil) for chemical profiling.

Qualitative analysis was performed using a TRACE™ 1610 gas chromatograph (Thermo Scientific) equipped with a TG-5SilMS capillary column (0.25 mm × 30 m, 0.25 µm) and coupled to a TSQ™ 9610 mass spectrometer with an advanced electron ionization source (AEI), operating at 70 eV in full scan mode (m/z 40–400). Ion source and transfer line temperatures were maintained at 250 °C.

Helium was used as the carrier gas at a pressure of 9.246 psi, linear velocity of 38.7 cm/s, and a column flow rate of 1.0 mL/min. The purge flow was set at 5.0 mL/min. Samples were introduced via autosampler in split mode, with the injector temperature maintained at 250°C.

The oven temperature program initiated at 50°C (held for 3 min), followed by a ramp to 200 °C over 15 min, then to 230 °C over 2.5 min, with a final hold at 230°C for 1 min. Total run time was 21.5 minutes.

GC-MS analysis was performed using helium (He) as the carrier gas, with a pressure of 9.246 psi and a linear flow rate of 38.7 cm s^{-1}. The flow rate of the carrier gas in the column was of 1.0 mL min^{-1} and the purge flow was 5.0 mL min^{-1}. The injection type was automatic (autosampler) in Split mode and the injector temperature was of 250ºC.

The oven heating program started at a temperature of 50ºC, held for 3 minutes; increased from 50-200ºC in 15 minutes; then increased from 200-230ºC in 2.5 minutes and finally held at 230ºC for 1 minute. The total run time was of 21.5 minutes.

Samples were diluted in dichloromethane to a final concentration of 1 mg/mL, filtered, and injected. Chromatographic peaks were identified via mass spectral comparison with the NIST database (147,198 compounds).

3 Results and discussion

In the larval reduction assay using different concentrations of *Lippia alba* essential oil (EO), a significant reduction in larval count was observed in the treated groups when compared to the control, except for the 10 mg/mL group. The most pronounced reductions were recorded at higher concentrations (Table 1).

Table 1 – Mean ± Standard Deviation of larval counts and percentage of third-stage larval reduction of gastrointestinal nematodes in goats, recovered via quantitative coproculture following exposure to various concentrations of *Lippia alba* essential oil

Treatment	Number of larvae	% of larvae reduction
Water control	2218 ± 433 [a]	–
Lippia alba 10 mg/mL	1559 ± 547 [a]	29.7
Lippia alba 25 mg/mL	454 ± 549 [b]	79.5
Lippia alba 50 mg/mL	271 ± 229 [b]	87.8
Lippia alba 100 mg/mL	225 ± 292 [b]	89.8

(*) Different lowercase letters in the same column indicate statistically significant differences ($p < 0.05$)
Source: research data

A decreasing trend in larval recovery was evident with increasing concentrations of *L. alba* EO, although no statistically significant differences were observed among the 25, 50, and 100 mg/mL treatments. The control group displayed larval counts consistent with expectations, based on the egg count (EPG) of the pooled sample, which totaled 2800 eggs.

The group treated with 10 mg/mL of *L. alba* EO exhibited no statistically significant difference

compared to the control. In contrast, treatment with 25 mg/mL resulted in a statistically significant reduction in larval counts relative to both the control and the 10 mg/mL groups. Although lower larval counts were recorded in the 50 and 100 mg/mL treatment groups, these differences were not statistically significant when compared to the 25 mg/mL treatment ($p > 0.05$).

The larval reduction observed in the in vitro assays, particularly at the concentration of 25 mg/mL of *L. alba* EO, indicates a promising effect against gastrointestinal nematodes. Nonetheless, it is important to emphasize that clinical efficacy in moderate to severe infections depends on multiple factors, including the compound's bioavailability within the gastrointestinal tract, the host's immune response, and the initial parasite burden. Therefore, although the in vitro results suggest potential for parasite control, in vivo studies are essential to determine whether such reductions are sufficient to achieve effective clinical control under real infection conditions.

Microscopic identification of the third-stage larvae (L3) revealed *Haemonchus spp*. as the most prevalent genus (56.8%), followed by *Trichostrongylus spp*. (42.9%) and *Oesophagostomum spp*. (0.3%). These findings align with previous studies reporting the predominance of these genera in goats (Dias *et al*., 2022; Maia; Mattos, 2020; Oliveira *et al*., 2018).

Temperature and humidity are critical factors influencing the hatching and development of L3 larvae. The seasonal timing of the present experiment, conducted during spring, likely influenced the genus distribution observed, particularly favoring the predominance of *Haemonchus spp*., which thrives under warmer conditions, as opposed to *Trichostrongylus* and *Oesophagostomum*, which prefer cooler temperatures and higher humidity (Amarante, 2015; Wilmsen *et al*., 2014). These results highlight the influence of seasonality on the population dynamics of gastrointestinal nematodes, corroborating the observations reported by Amarante (2015).

The essential oil of *L. alba* is known to exhibit significant compositional variability, influenced by both genetic factors and environmental conditions, such as UV radiation, soil nutrients, seasonality, light exposure, and the developmental stage of the plant (Barbosa *et al*., 2023; Neves; Santana; Krepsky, 2021).

Numerous studies support the anthelmintic potential of essential oils and their major constituents, both in vitro and in vivo. Compounds such as monoterpenes (e.g., limonene, α-pinene), sesquiterpenes (e.g., β-caryophyllene), alcohols (e.g., geraniol), phenols (e.g., carvacrol), and aldehydes (e.g., citronellal) have demonstrated promising antiparasitic effects and may represent viable alternatives for parasite control (Abidi *et al*., 2018).

The major components identified in the *L. alba* essential oil used in this study were E-citral (geranial) (23.76%), Z-citral (neral) (20.46%), carvone (10.92%), and limonene (6.40%), accounting for 61.54% of the total composition. In total, 25 compounds were identified, with 5 remaining unidentified. The complete chemical profile is presented in Table 2.

Table 2 – Retention time, chemical composition, literature Kovats index (KL), and percentages of identified components (%) in the essential oil of *Lippia alba*

	TR	Compounds	IK	%
1	8.71	α-Tujene	930	0.65
2	8.88	α-Pinene	939	1.34
3	9.61	Sabinene	975	1.92
4	9.73	6-Methyl-5-hepten-2-one	985	1.05
5	9.83	β-Myrcene	990	5.39
6	10.51	p-Cimene	1024	5.20
7	10.60	**Limonene**	1029	**6.40**
8	10.84	E-β-Ocimene	1050	1.90
9	11.11	γ-Terpinene	1059	4.24
10	11.72	Linalool	1096	1.20
11	12.94	Z-Isocitral (isoneral)	1164	0.66

12	12.94	Unidentified		0.51
13	13.03	Unidentified	-	0.90
14	14.01	**Z-Citral (neral)**	1238	**20.46**
15	14.14	**Carvone**	1243	**10.92**
16	14.42	**E-Citral (geranial)**	1267	**23.76**
17	15.57	Piperitone	1343	0.23
18	15.89	Geranial acetate	1381	0.57
19	16.26	Unidentified	-	0.78
20	16.76	E-Caryophyllene	1419	4.79
21	17.21	α-Humulene	1454	0.80
22	17.54	Germacrene D	1481	3.33
23	18.29	Unidentified	-	0.36
24	18.54	Unidentified	-	0.93
25	18.88	Caryophyllene oxide	1583	1.71
		Total		100.00

Source: research data

Based on these results, *Lippia alba* can be classified as a carvone–citral chemotype, with citral (44.22%) comprising a mixture of the isomers geranial and neral (Santos *et al*., 2016). The carvone chemotype has demonstrated antimicrobial efficacy across several experimental studies, particularly against Gram-positive bacteria. In the study by Barbieri *et al*. (2014), commercially sourced carvone exhibited 100% efficacy against *Haemonchus contortus* eggs at a concentration of 2.08 mg/mL. Carvone is known for its bactericidal, fungicidal, and repellent properties (Soares; Tavares-Dias, 2013), making it a promising candidate in the development of anthelmintic compounds.

Barbosa *et al*. (2023) also evaluated the in vitro anthelmintic activity of *L. alba* essential oil against different developmental stages of *H. contortus*, including eggs and isolated adult worms. The analyzed oil contained citral (49.8%) and carvone (12.8%) as major constituents. At a concentration of 1 mg/mL, 95% inhibition of egg hatching was observed, and treatment with 0.5 mg/mL reduced larval motility by 90% within 12 hours. Concentrations of 1.0 and 2.0 mg/mL led to complete inhibition of both egg hatching and larval motility within just 3 hours. Although these findings reinforce the bioactivity of L. alba essential oil, methodological differences, particularly regarding parasite developmental stages and tested concentrations, prevent direct comparisons with the present study, which focused on larval development inhibition (egg to L3 stage) using higher concentrations. Nonetheless, these data highlight the significance of these chemical constituents.

The inhibitory effect of the compounds geranial and neral was also demonstrated in assays by Macedo *et al*. (2019), which assessed their antiparasitic activity on *Haemonchus sp*. eggs. The study reported a 98.4% inhibition of egg hatching, highlighting the therapeutic potential of these components for controlling gastrointestinal nematodes. These findings support the importance of the chemical composition of *L. alba* essential oil, particularly the high presence of geranial and neral, in the observed anthelmintic effects.

Geranial, the main compound identified in the present analysis (23.76%), was also examined by Macedo *et al*. (2015) in assays using essential oil from *Cymbopogon citratus*, which contained 57.3% geranial. In that study, treatment against *H. contortus* resulted in 38.5% efficacy. Neral, the second most abundant compound (20.46%), has not yet been fully studied alone against nematode populations, and its specific effects remain poorly understood in the literature.

The fourth most abundant component found in L. alba essential oil was limonene (6.40%), a monocyclic monoterpene (4-isoprenyl-1-methylcyclohexene) with a characteristic citrus aroma. Limonene has a variety of pharmacological properties, including antimicrobial, antifungal, and antiparasitic activities (Hu *et al*., 2018). It may play a significant role in inhibiting larval hatching in gastrointestinal nematodes of goats. Macedo *et al*. (2010), using essential oil from Eucalyptus

staigeriana (where limonene was the main compound at 28.82%), reported a 61.4% reduction in parasitic load after eight days of treatment.

These findings, when compared to existing literature, reveal considerable variation in the main constituents of L. alba essential oil. This variability can be linked to environmental factors such as climate, soil conditions, plant age, atmospheric pollution, altitude, temperature, water availability, and nutrient levels. Additionally, harvesting practices, drying methods, and storage conditions can greatly influence the production of secondary metabolites (André *et al*., 2018; Mottin *et al*., 2019).

The study of isolated compounds from essential oils is a rapidly expanding field across various scientific disciplines. However, when it comes to *L. alba* and its isolated constituents for anthelmintic use in goats, the existing literature is limited and outdated. Further research is crucial, given the promising potential of these compounds for preventing and controlling helminth infections (Doan *et al*., 2020). Isolated compounds can target multiple receptors within nematodes at both egg and larval stages, thereby reducing the development of resistance often linked to synthetic anthelmintics (André *et al*., 2018).

In assays examining the hatching rate of helminth eggs, essential oils have been shown to hinder early larval development (Macedo *et al*., 2011), possibly through the action of enzymes such as lipases, proteases, β-glucosidases, chitinases, and leucine aminopeptidases, which are involved in degrading the eggshell during hatching (Molan; Faraj, 2010). The activity of the main compounds studied here (carvone and citral) may relate to their capacity to inhibit acetylcholinesterase (Kurt *et al*., 2017), a mechanism commonly associated with organophosphates (Ross *et al*., 2013).

Eggs of *H. contortus* have a lipid-protein outer layer (Mansfield; Gamble; Fetterer, 1992), and the terpenes carvone and citral are thought to extract or disrupt these structural components (Krishnaiah *et al*., 2006). This could explain the reduction in larval counts observed in this study, as embryonic development becomes impaired. Additionally, studies indicate that citral is more effective in helminth control than carvone, as it increases eggshell permeability and prevents larval formation, whereas carvone exhibits a more delayed effect (Cao *et al*., 2021).

4 Conclusions

The results indicate that the essential oil of *Lippia alba* demonstrated in vitro efficacy in reducing gastrointestinal nematode larvae in goats, starting at a concentration of 25 mg/mL, with *Haemonchus sp*. and *Trichostrongylus sp*. identified as the predominant genera. The chemical characterization revealed that the major constituents of the oil were E-Citral, Z-Citral, Carvone, and Limonene, all compounds associated with the observed biological activity. These findings underscore the potential of *L. alba* essential oil as a promising alternative for controlling gastrointestinal nematodes, supporting the need for further research to evaluate its practical application in vivo and in field conditions.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Ethical considerations

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Contributions to the article

MORAIS, I. O. S.: Conception or design of the study/research; data collection, analysis, and/or interpretation; drafting and writing of the manuscript; overall supervision and coordination of the project or study. **TAVARES, F. F.:** Data collection, analysis, and/or interpretation; drafting and writing of the manuscript. **LIMA, S. M.:** Data collection, analysis, and/or interpretation; drafting and writing of the manuscript. **SILVA, F.:** Data collection, analysis, and/or interpretation; drafting and writing of the manuscript. **CATUNDA JÚNIOR, F. E. A.:** Data collection, analysis, and/or interpretation; drafting and writing of the manuscript. **PERINOTTO, W. M. S.:** Conception or design of the study/research; data collection, analysis, and/or interpretation; drafting and writing of the manuscript; Critical revision with significant intellectual contribution; overall supervision and coordination of the project or study. All authors participated in the writing, discussion, reading, and approval of the final version of the manuscript.

References

ABIDI, A.; SEBAI, E.; DHIBI, M.; ALIMI, D.; REKIK, M.; B'CHIR, F.; MAIZELS, R. M.; AKKARI, H. Chemical analyses and anthelmintic effects of *Artemisia campestris* essential oil. **Veterinary Parasitology**, v. 263, p. 59-65, 2018. DOI: https://doi.org/10.1016/j.vetpar.2018.10.003.

AMARANTE, A. F. T. **Os parasitos de ovinos.** São Paulo: Editora Unesp, 2015. In Portuguese.

ANDRÉ, W. P. P.; RIBEIRO, W. L. C.; OLIVEIRA, L. M. B.; MACEDO, I. T. F.; RONDON, F. C. M.; BEVILAQUA, C. M. L. Óleos essenciais e seus compostos bioativos no controle de nematoides gastrintestinais de pequenos ruminantes. **Acta Scientiae Veterinariae**, v. 46, e1522, 2018. Available at: https://seer.ufrgs.br/index.php/ActaScientiaeVeterinariae/article/view/81804/48969. Accessed on: 01 out. 2024. In Portuguese.

BALDISSERA, M. D.; SOUZA, C. F.; MOURÃO, R. H. V.; SILVA, L. V. F.; MONTEIRO, S. G. Trypanocidal action of *Lippia alba* and *Lippia origanoides* essential oils against *Trypanosoma evansi* in vitro and in vivo used mice as experimental model. **Journal Parasitic Diseases**, v. 41, p. 345-351, 2017. DOI: https://doi.org/10.1007/s12639-016-0800-7.

BARBIERI, A. M. E.; LOUVANDINI, H.; ARAÚJO, R. C.; KATIKI, L. M. 2014. Potencial anti-helmíntico de óleos essenciais em nematoides gastrintestinais de ovinos. In: CONGRESSO INTERINSTITUCIONAL DE INICIAÇÃO CIENTÍFICA – CIIC, 8., 2014, Campinas. **Proceedings [...]**. Campinas, 2014. Available at: https://www.iac.sp.gov.br/areadoinstituto/ciiciac/resumo2014/RE14704.pdf. Accessed on: 01 out. 2024. In Portuguese.

BARBOSA, M. L. F.; RIBEIRO, W. L. C.; ARAÚJO FILHO, J. V.; PEREIRA, R. C. A.; ANDRÉ, W. P. P.; MELO, A. C. F. L.; CASTELO-BRANCO, D. S. C. M.; MORAIS, S. M.; OLIVEIRA, L. M. B.; BEVILAQUA, C. M. L. *In vitro* anthelmintic activity of *Lippia alba* essential oil chemotypes against *Haemonchus contortus*. **Experimental Parasitology**, v. 244, 108439, 2023. DOI: https://doi.org/10.1016/j.exppara.2022.108439.

BRAZIL. **Instrução Normativa nº 46: Regulamento técnico para os sistemas orgânicos de produção animal e vegetal**. Brasília: Presidência da República, 2011. Available at: https://www.gov.br/agricultura/pt-br/assuntos/sustentabilidade/organicos/legislacao/portugues/instrucao-normativa-no-46-de-06-de-outubro-de-2011-producao-vegetal-e-animal-regulada-pela-in-17-2014.pdf/view. Accessed on: 11 ago. 2025. In Portuguese.

CAO, J. J.; LIU, H.; WANG, Y.; ELE, X.; HEYANG, J.; YAO, J.; XIA, F.; ZHAO, Y.; CHEN, X. Antimicrobial and antivirulence efficacies of citral against foodborne pathogen *Vibrio parahaemolyticus* RIMD221033. **Food Control**, v. 120, e107507, 2021. DOI: https://doi.org/10.1016/j.foodcont.2020.107507.

CHNITER, M.; DHAOUI, A.; HOUIDHEG, A.; ATIGUI, M.; HAMMADI, M. Socio-economic aspects and farming practices of goats in Southern Tunisia. **Tropical Animal Health and Production**, v. 56, n. 7, art. 220, 2024. DOI: https://doi.org/10.1007/s11250-024-04063-2.

DIAS, A. M. A.; MONTEIRO, K. J. L.; SOUSA JÚNIOR, A.; NUNES, J. F.; CARDOSO, J. F. S.; PAULA, N. R. O.; VIEIRA, R. J. Prevalência de helmintos gastrintestinais em caprinos da região do Baixo Parnaíba – PI. **Research, Society and Development**, v. 11, n. 7, e59011730342, 2022. DOI: https://doi.org/10.33448/rsd-v11i7.30342. In Portuguese.

DOAN, H.V.; SOLTANI, E., INGELBRECHT, J., SOLTANI, M. Medicinal herbs and plants: potential treatment of monogenean infections in fish. **Reviews in Fisheries Science and Aquaculture**, v. 28, n. 2, p. 260-282, 2020. DOI: https://doi.org/10.1080/23308249.2020.1712325.

ESTEBAN-BALLESTEROS, M.; ROJO-VÁSQUEZ, F. A.; SKUCE, P. J.; MELVILLE, L.; GONZÁLEZ-LANZA, C.; MARTÍNEZ-VALLADARES, M. Quantification of resistant alleles in the β-tubulin gene of field strains of gastrointestinal nematodes and their relation with the faecal egg count reduction test. **BMC Veterinary Research**, v. 71, n. 13, p. 1-8, 2017. DOI: https://doi.org/10.1186/s12917-017-0992-9

FTHENAKIS, G.C.; PAPADOPOULOS, E. Impact of parasitism in goat production. **Small Ruminant Research**, v. 163, p. 21-23, 2018. DOI: https://doi.org/10.1016/j.smallrumres.2017.04.001.

HU, J.; JIA, M.; ZHU, L. Chemical composition and antimicrobial activities of essential oil from *Wedelia urticifolia* growing wild in Hunan Province, China. **Natural Product Research**, v. 33, n. 18, p. 1-4, 2018. DOI: https://doi.org/10.1080/14786419.2018.1460830.

KRISHNAIAH, Y. S. R.; AL-SAIDAN, S. M.; CHANDRASEKHAR, D. V.; RAMA, B. Effect of nerodilol and carvone on in vitro permeation of nicorandil across rat epidermal membrane. **Drug Development and Industrial Pharmacy**, v. 32, n. 4, p. 423-435, 2006. DOI: https://doi.org/10.1080/03639040500528939.

KURT, B. Z.; GAZIOGLU, I.; DAG, A.; SALMAS, R. E.; KAYIK, G.; DURDAGI, S.; F.; SONMEZ, F. Synthesis, anticholinesterase activity and molecular modeling study of novel carbamate-substituted thymol/carvacrol derivatives. **Bioorganic and Medicinal Chemistry**, v. 25, n. 4, p. 1352-1363, 2017. DOI: https://doi.org/10.1016/j.bmc.2016.12.037.

LEÓN, J. C. P.; DELGADO, N. U.; FLOREZ, A. A. Prevalence of gastrointestinal parasites in cattle and sheep in three municipalities in the Colombian Northeastern Mountain. **Veterinary World**, v. 12, n. 1, p. 48-54, 2019. DOI: https://doi.org/10.14202/vetworld.2019.48-54.

MACEDO, I. T. F.; BEVILAQUA, C. M. L.; OLIVEIRA, L. M. B.; CAMURÇA-VASCONCELOS, A. L. F.; VIEIRA, L. S.; OLIVEIRA, F. R.; QUEIROZ-JUNIOR, E. M.; TOMÉ, A. R.; NASCIMENTO, N. R. F. Anthelmintic effect of *Eucalyptus staigeriana* essential oil against goat gastrointestinal nematodes. **Veterinary Parasitology**, v. 173, n. 1-2, p. 93-98, 2010. DOI: https://doi.org/10.1016/j.vetpar.2010.06.004.

MACEDO, I. T. F.; BEVILAQUA, C. M. L.; OLIVEIRA, L. M. B.; CAMURÇA-VASCONCELOS, A. L. F.; VIEIRA, L. S.; AMÓRA, S. S. A. Evaluation of *Eucalyptus citriodora* essential oil on goat gastrointestinal nematodes. **Revista Brasileira de Parasitologia Veterinária**, v. 20, n. 3, p. 223-227, 2011. DOI: https://doi.org/10.1590/S1984-29612011000300009.

MACEDO, I. T. F.; OLIVEIRA, L. M. B.; RIBEIRO, W. L. C.; SANTOS, J. M. L.; SILVA, K. C.; ARAÚJO FILHO, J. V.; CAMURÇA-VASCONCELOS, A. L. F.; BEVILAQUA, C. M. L.

Anthelmintic activity of *Cymbopogon citratus* against *Haemonchus contortus*. **Revista Brasileira de Parasitologia Veterinária**, v. 24, n. 3, p. 268-275, 2015. DOI: https://doi.org/10.1590/S1984-29612015059.

MACEDO, I. T. F.; DE OLIVEIRA, L. M. B.; ANDRÉ, W. P. P.; ARAÚJO FILHO, J. V.; SANTOS, J. M. L.; RONDON, F. C. M.; RIBEIRO, W. L. C.; CAMURÇA-VASCONCELOS, A. L. F.; OLIVEIRA, E. F.; PAULA, H. C. B.; BEVILAQUA, C. M. L. Anthelmintic effect of *Cymbopogon citratus* essential oil and its nanoemulsion on sheep gastrointestinal nematodes. **Revista Brasileira de Parasitologia Veterinária**, v. 28, n. 3, p. 533-527, 2019. DOI: https://doi.org/10.1590/s1984-29612019065.

MACEDO, L. O.; SILVA, S. S.; ALVES, L. C.; CARVALHO, G. A.; RAMOS, R. A. N. An overview of anthelmintic resistance in domestic ruminants in Brazil. **Ruminants**, v. 3, n. 3, p. 214-232, 2023. DOI: https://doi.org/10.3390/ruminants3030020.

MAIA, D.; MATTOS, M. J. T. Nematodeoses gastrintestinais em bovinos no Brasil: revisão de artigos publicados no período de 2012 a 2020**. Revista Agrária Acadêmica**, v. 3, n. 3, p. 296-307, 2020. DOI: https://doi.org/10.32406/v3n32020/296-307/agrariacad. In Portuguese.

MANSFIELD, L. S.; GAMBLE, H. R.; FETTERER, R. H. Characterization of the eggshell of *Haemonchus contortus* - I. Structural components. **Comparative Biochemistry and Physiology Part B: Comparative Biochemistry**, v. 103, n. 3, p. 681-686, 1992. DOI: https://doi.org/10.1016/0305-0491(92)90390-d.

MOLAN, A.-L.; FARAJ, A. M. The effects of condensed tannins extracted from different plant species on egg hatching and larval development of *Teladorsagia circumcincta* (nematoda:Trichostrongylidae). **Folia Parasitologica**, v. 57, p. 62-68, 2010. DOI: https://doi.org/10.14411/fp.2010.008.

MOTTIN, V. D.; CRUZ, J. F.; TEIXEIRA NETO, M. R.; MARISCO, G.; FIGUEREDO, J. S.; SOUSA, L. S. Efficacy, toxicity, and lethality of plants with potential anthelmintic activity in small ruminants in Brazil. **Revista Brasileira de Saúde e Produção Animal**, v. 20, e0232019, 2019. DOI: https://doi.org/10.1590/S1519-99402002320 19.

NEVES, D. S. C.; SANTANA, G. N.; KREPSKY, P. B. Variação intraespecífica na composição e teor do óleo essencial de *Lippia thymoides*. **Revista Fitos**, v. 15, p. 192- 203, 2021. DOI: https://doi.org/10.32712/2446-4775.2021.1062. In Portuguese.

OLIVEIRA, D. A. S.; BRITO, R. L. L.; NEVES, M. R. M.; SOUSA, M. M.; MIRANDA, R. R. C.; MOURÃO, A. E. B.; CAVALCANTE, A. C. R.; VIEIRA, L. S. Parasitos gastrintestinais em caprinos no munícipio de Quixadá, Ceará. **Pesquisa Veterinária Brasileira**, v. 38, n. 8, p. 1505-1510, 2018. DOI: https://doi.org/10.1590/1678-5150-PVB-4828. In Portuguese.

OLIVEIRA, G. T.; FERREIRA, J. M. S.; ROSA, L. H.; SIQUEIRA, E. P.; JOHANN, S.; LIMA, L. A. R. S. *In vitro* antifungal activities of leaf extracts of *Lippia alba* (Verbenaceae) against clinically important yeast species. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 47, n. 2, p. 247-250, 2014. DOI: https://doi.org/10.1590/0037-8682-0008-2013.

OLIVERO-VERBEL, J.; BARRETO-MAYA, A.; BERTEL-SEVILLA, A.; STASHENKO, E. E. Composition, anti-quorum sensing and antimicrobial activity of essential oils from *Lippia alba*. **Brazilian Journal of Microbiology**, v. 45, n. 3, p. 759-767, 2014. DOI: https://doi.org/10.1590/S1517-83822014000300001.

PAVLOVIĆ, D. M.; KRALJEVIĆ, T. G.; PAVIĆ, R.; MRDA, J. Determination of anthelmintic pharmaceuticals in wastewater by solid-phase extraction and thin-layer chromatography. J. **Planar**

Chromatography, v. 32, n. 5, p. 421-429, 2019. DOI: https://doi.org/10.1556/1006.2019.32.5.10.

RODRIGUES, M. L. A.; BITTENCOURT, V. R. E. P.; ANJOS, D. H. S.; CASTRO, A. A. Efeito do fungo *Metarhizium anisopliae* sobre os estádios pré-parasíticos de Cyathostominae (Nematoda: Strongylidae). **Ciência Rural**, v. 26, n. 2, p. 301-303, 1996. DOI: https://doi.org/10.1590/S0103-84781996000200023. In Portuguese.

ROSS, S. M.; MCMANUS, I. C.; HARRISON, V.; MASON, O. Neurobehavioral problems following low-level exposure to organophosphate pesticides: a systematic and meta-analytic review. **Critical Reviews in Toxicology**, v. 43, n. 1, p. 21-44, 2013. DOI: https://doi.org/10.3109/10408444.2012.738645.

SALGADO, J. A.; SANTOS, C. P. Overview of anthelmintic resistance of gastrointestinal nematodes of small ruminants in Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 25, n. 1, p. 3-17, 2016. DOI: https://doi.org/10.1590/S1984-29612016008

SANTOS, N. O.; PASCON, R. C.; VALLIM, M. A.; FIGUEIREDO, C. R.; SOARES, M. G.;LAGO, J. H. G.; SARTORELLI, P. Cytotoxic and antimicrobial constituents from the essential oil of *Lippia alba* (Verbenaceae). **Medicines**, v. 3, n. 3, 22, 2016. DOI: https://doi.org/10.3390/medicines3030022.

SILVA, F. F.; BEZERRA, H. M. F. F.; FEITOSA T, F.; VILELA, V. L. R. Nematode resistance to five anthelmintic classes in naturally infected sheep herds in Northeastern Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 27, n. 4, p. 423-429, 2018. DOI: https://doi.org/10.1590/S1984-296120180071.

SOARES, B. V.; TAVARES-DIAS, M. Espécies de *Lippia* (Verbenaceae), seu potencial bioativo e importância na medicina veterinária e aquicultura. **Biota Amazônia**, v. 3, p. 109-123, 2013. Available at: https://www.alice.cnptia.embrapa.br/alice/handle/doc/965697. Accessed on: 31 jul. 2025. In Portuguese.

SOARES, S. C. P.; REIS, A. C.; CASTRO, R. L. P.; PIRES FILHO, P. C. S.; CABRAL, C. S. M.; DINIZ JÚNIOR, D. O.; COSTA, C. B.; PINHEIRO, D. M. C.; PINHEIRO, Y. S. A.; BUNA, N. H. S.; COSTA JÚNIOR, L. M.; BRITO, D. R. B. Resistance of gastrointestinal nematoids of goats and sheep to the antihelmintics levamisol, ivermectin and albendazole. **Brazilian Animal Science,** v. 24, e-75316E, 2023. DOI: https://doi.org/10.1590/1809-6891v24e-75316P

SNOW, D. D.; CASSADA, D. A.; BISWAS, S.; MALAKAR, A.; D'ALESSIO, M.; CARTER, L. J.; JOHNSON, R. D.; SALLACH, J. B. Detection, occurrence and fate of emerging contaminants in agricultural environments. **Water Environment Research**, v. 91, n. 10, p. 1103-1113, 2019. DOI: https://doi.org/10.1002/wer.1204.

UENO, H.; GONÇALVES, P. C. **Manual para diagnóstico das helmintoses de ruminantes**. 4. ed. Tokyo: Japan International Cooperation Agency, 1998. In Portuguese.

VERA, S. S.; ZAMBRANO, D. F.; MÉNDEZ-SANCHEZ, S. C.; RODRÍGUEZ-SANABRIA, F.; STASHENKO, E. E.; LUNA, J. E. D. Essential oils with insecticidal activity against larvae of *Aedes aegypti* (Diptera: Culicidae). **Parasitology Research**, v. 113, p. 2647-2654, 2014. DOI: https://doi.org/10.1007/s00436-014-3917-6.

WILMSEN, M. O.; SILVA, B. F.; BASSETTO, C. C.; AMARANTE, A. F. T. Gastrointestinal nematode infections in sheep raised in Botucatu, state of São Paulo, Brazil. **Revista Brasileira de Parasitologia Veterinária**, v. 23, n. 3, p. 348-354, 2014. DOI: https://doi.org/10.1590/S1984-29612014058.