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Research Article

Acaricidal Effects of *Melaleuca cajuputi* Essential Oil on *Rhipicephalus sanguineus* Ticks in Dogs

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Abstract | The *Rhipicephalus sanguineus* tick, a common parasite of dogs, poses significant health risks to both canines and humans. This study assessed the impact of *Melaleuca cajuputi* essential oil on the larval development and reproduction of engorged female ticks of this species. The essential oil was obtained through steam distillation, and its active constituents were analysed using gas chromatography-mass spectrometry (GC-MS). To evaluate its effectiveness in larval mortality and reproductive inhibition in ticks, adult immersion and larval packet assays were performed. Chemical analysis revealed 45 components, comprising over 97.66% of the total constituents, with major compounds including α-Terpineol, Eucalyptol, β-Linalool, β-Selinene, and β-Caryophyllene. The lethal effects of *Melaleuca cajuputi* essential oil on larvae were assessed using LD50, LD90, and LD99 values of 1.27, 8.96, and 43.95 mg/mL, respectively. The oil also reduced egg-laying capacity, with a 42.82±2.21% reduction at the LD50 concentration for larvae. These findings suggest that *Melaleuca cajuputi* essential oil has strong in vitro larvicidal potential and can negatively impact the reproductive capacity of *Rhipicephalus sanguineus* ticks.

Keywords | Acaricide, *Melaleuca cajuputi,* Essential oils, *Rhipicephalus sanguineus,* Ticks, Dogs

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INTRODUCTION

In recent years, pet ownership has been on the rise, with dogs being among the most popular choices. Consequently, concerns about pet health have also increased. Ectoparasitic infestations are frequently observed in pets, particularly in dogs kept in free-ranging or semi-free-ranging environments, which makes them more vulnerable to parasitic diseases. Among these parasites, *Rhipicephalus sanguineus* ticks are especially significant, as they are one of the most common ectoparasitic arthropods affecting dogs (James and Harwood, 1979). Ticks function as vectors for several serious diseases in dogs, including *Ehrlichia canis*, *Anaplasma platys, Hepatozoon canis, and Cercopithifilaria* spp. (Sainz *et al*., 2015). These parasitic arthropods play a crucial role in disease transmission, acting as intermediaries that spread infections among various animal species and pose a risk to humans by transmitting diseases such as rodent plague, viral encephalitis, typhus fever, and protozoal infections (Piotrowski and Rymaszewska, 2020).

To manage tick infestations in dogs, a range of chemical parasiticides, including ivermectin, amitraz, fipronil, and pyrethroids, are widely used for tick elimination. Although these chemical treatments are effective, they come with high costs, and their overuse or misuse can harm pets, contribute to resistance in *Rhipicephalus sanguineus* tick populations, and leave toxic residues in the environment, endangering both animals and humans (Cunha *et al*., 2017). Studies have confirmed that ticks have developed resistance to several acaricides globally (Becker *et al*., 2019; Higa *et al*., 2020). As a result, there is a growing need to explore new treatment strategies and tick control measures for dogs. Research has highlighted that bioactive compounds derived from medicinal plants offer promising alternatives to synthetic chemicals for managing both tick larvae and adults (Silva *et al*., 2023; Pereira *et al*., 2023).

Herbal essential oils have been widely utilized as medicinal agents in both veterinary and human medicine, with *Melaleuca cajuputi* tree being a prominent example. This essential oil is rich in bioactive compounds, including terpinen-4-ol, α-terpineol, eucalyptol, α-pinene, limonene, linalool, and guaiol (Isah *et al*., 2023). Studies have shown that these compounds exhibit antimicrobial effects by inhibiting bacterial growth (Shahbazi *et al*., 2015), possess antifungal properties (Smid *et al*., 1995), and have insecticidal capabilities (Lee *et al*., 1997). In addition, they have been found effective against pests such as mites (Yang *et al*., 2014), and *Amblyomma hebraeum* ticks (Mkolo *et al*., 2011). Due to these properties, *Melaleuca cajuputi* essential oil holds significant potential as an acaricidal agent for managing ticks on dogs. This study seeks to evaluate its efficacy in eliminating *Rhipicephalus sanguineus* ticks that infest dogs.

MATERIALS AND METHODS

Essential Oils Extraction

Melaleuca cajuputi essential oil was obtained through the steam distillation process. Following harvesting, the leaves were dried at 40°C for 60 hours in a vegetable drying oven until a stable weight was achieved. Once dried, the leaves were finely ground into powder using a grinder, resulting in a particle size of approximately 1–2 mm. A total of 150 g of *Melaleuca cajuputi* leaf powder was combined with 1300 ml of distilled water and placed into the 1000 ml flask of a Clevenger apparatus for essential oil distillation. The mixture was heated on an electric stove at 100°C, and the steam flow rate was maintained at approximately 1.5–2.0 ml/min. The distillation process continued until the essential oil yield stabilized, which took approximately 2 hours. The extracted *Melaleuca cajuputi* essential oil appeared as a pale-yellow liquid, lighter than water, with a distinct pleasant aroma. To eliminate residual moisture, the oil was treated with sodium sulfate. It was then thoroughly mixed, protected from oxidation by covering it with aluminum foil, and stored at 4°C.

The composition and relative content of volatile compounds in the essential oils were analysed using gas chromatography–mass spectrometry (GC–MS) on an Agilent 6890N system with an HP5-MS column and a 5973 Inert MS detector. The temperature program started at 50°C (2 min), ramped at 5°C/min to 150°C, 10°C/min to 200°C, and 20°C/min to 300°C (held 5 min). Helium (1 ml/min) was the carrier gas; injector and detector were set at 250°C with a split ratio of 30:1. Essential oils (10 µl) were diluted in 1 ml hexane, and 0.2 µl was injected. Mass spectra (40–400 m/z, 70 eV) and linear retention indices were compared with NIST, Wiley, and published data for compound identification (Adams, 2007).

Preparation of the Dilutions

Solutions of *Melaleuca cajuputi* essential oil were formulated at concentrations of 20, 10, 5, 2.5, 1.25, 0.625, and 0.3125 mg/ml, with 96% ethanol serving as the solvent. For the controls, 96% ethanol was used as the negative control, while pyrethroid (Hanvet, Vietnam) served as the positive control. To assess the 50% lethal dose (LD50) of pyrethroid for tick larvae, it was mixed with distilled water to reach final concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 µg/ml.

Identification of Engorged Female *Rhipicephalus sanguineus*

Engorged female ticks were identified morphologically under a stereomicroscope based on key taxonomic characteristics. Identification followed the dichotomous keys described by Walker *et al*. (2003), which include the presence of a hexagonal basis capitulum with sharply angulated posterior margins, short palps, and a reddish-brown scutum that only partially covers the dorsum in females. Additional distinguishing features of engorged females include an enlarged, soft, and grayish body due to blood feeding, which facilitates separation from other stages and species. The morphology of the spiracular plates, festoons, and coxal spurs was also used to confirm species identity.

Ticks Preparation

Engorged female *Rhipicephalus sanguineus* ticks were obtained from client-owned dogs with natural infestations that had not undergone any tick-specific treatments. The dogs were brought to the veterinary clinic of Tra Vinh University for examination and treatment, and tick collection was performed with the owners' informed consent. The study procedures were approved by the Animal Care and Use Committee of Tra Vinh University (License No. 06/2024/HD-HDKH&DT-DHTV). Ticks were kept in cloth-covered jars at 27°C and 80% humidity until egg-laying was complete. Eggs were maintained under the same conditions until hatching, and five-day-old larvae were selected for essential oil testing.

Testing the Larvicidal Activity of Essential Oil

Five-day-old larvae (200 larvae per cloth bag) were immersed in petri dishes with 2 ml of essential oil solutions. After saturation, they were incubated at 27°C and 80% humidity for 24 hours. Survival was then examined under a microscope. The procedure was repeated five times, and mortality was calculated using the formula outlined by Castro *et al.* (2018).

$$Corrected\ mortality\ (\%) = \frac{(Test\ mortality\ (\%) - Control\ mortality\ (\%)) \times 100}{100 - Control\ mortality\ (\%)}$$

Testing the Adulticidal Activity of Essential Oil

Female ticks (≥3.5 mm) were selected based on mobility, health, and weight (120-170 mg). They were weighed, grouped, and individually submerged for 5 minutes in essential oil solutions at concentrations causing 50% larval mortality. After drying, ticks were kept at 27°C and 80% humidity until egg-laying was complete. Egg masses were weighed and stored until hatching. The experiment was repeated five times, and reproductive capacity was evaluated using Drummond *et al.* (1973) method. Key reproductive parameters, including the index of egg production (EPI), eggs hatched (EH), reduction in oviposition (RO), and efficiency of production (EP), were calculated as follows:

$$EPI = \frac{egg\ mass\ weight}{tick\ weight} \times 100$$

$$RO = \frac{control\ EPI - treatment\ EPI}{control\ EPI} \times 100$$

$$EH = \frac{egg\ mass\ weight \times \%\ hatched}{total\ tick\ weight}$$

$$EP = \frac{control\ EH - treatment\ EH}{control\ EH} \times 100$$

Statistical Analysis

The median lethal dose (LD_{50}) was determined using probit analysis with SPSS version 22.0. Differences in LD_{50} values were considered significant when their 95% confidence intervals did not overlap. To evaluate the effects of essential oils on tick reproduction parameters, one-way analysis of variance (ANOVA) was performed. Prior to conducting ANOVA, the data were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. Both assumptions were met. Tukey's test was used as the post-hoc test for multiple comparisons among treatment groups, as it is suitable when ANOVA assumptions are satisfied and helps control the family-wise error rate. Statistical significance was set at $P<0.05$. All results are presented as mean ± standard deviation (SD).

Table 1: The composition profile of *Melaleuca cajuputi* essential oils.

Constituents	Retention time (min)	Relative percentage (%)
o-Cymene	7.031	0.10
D-Limonene	7.191	0.38
Eucalyptol	7.280	5.76
γ-Terpinene	8.154	0.09
Terpinolene	9.133	0.10
β-Linalool	9.664	5.56
Camphor	11.442	0.08
δ-Terpineol	12.428	0.96
Menthol	12.770	0.24
4-Terpineol	12.873	2.66
α-Terpineol	13.617	41.85
n-Dodecane	13.839	0.06
cis-Geraniol	14.786	0.24
trans-Geraniol	15.922	1.60
δ-Elemene	19.598	0.07
Eugenol	20.257	0.24
α-Ylangene	21.051	0.74
α-Copaene	21.330	0.38
Geranyl acetate	21.534	0.65
β-Elemene	21.954	0.26
β-Caryophyllence	23.174	5.19
γ-Elemene	23.663	0.40
α-Guaiene	23.902	0.28
Guaia-6,9-diene	24.140	0.16
α-Humulene	24.685	3.76
4,5-Di-epi-aristolochene	25.313	0.18
γ-Selinene	25.487	0.71
γ-Gurjunene	25.618	0.94
α-Amorphene	25.747	1.52
β-Selinene	26.098	5.56
γ-Amorphene	26.301	0.38
α-Selinene	26.418	3.72
α-Muurolene	26.580	0.09
γ-Cadinene	26.750	1.04
β-Cadinene	27.940	0.14
α-Panasinsene	28.572	0.66
Caryophyllene	29.839	0.51
Guaiol	30.542	3.95
Humulene epoxide II	30.954	0.34
Rosifoliol	31.098	0.06
Selin-6-en-4α-ol	31.289	0.18
γ-Eudesmol	31.889	1.74

Agarospirol	32.176	0.19
β-Eudesmol	32.734	3.10
Bulnesol	33.182	0.84
Total		97.66

RESULTS

Extraction and Chemical Composition Analysis of Essential Oil

By applying the steam distillation technique, 2.2 ml of essential oil was extracted from 150 g of dried *Melaleuca cajuputi* leaf powder, resulting in a yield of 1.47%. This yield is consistent with previously reported values for *Melaleuca cajuputi*, although the composition may vary depending on factors such as plant origin and extraction methods. The composition of the essential oil's bioactive compounds is detailed in Table 1. GC-MS analysis identified 45 compounds, making up 97.66% of the total constituents. The major components of *Melaleuca cajuputi* essential oil included α-Terpineol (41.85%), Eucalyptol (5.76%), β-Linalool (5.56%), β-Selinene (5.56%), β-Caryophyllene (5.19%), Guaiol (3.95%), α-Humulene (3.76%), α-Selinene (3.72%), β-Eudesmol (3.10%), 4-Terpineol (2.66%), γ-Eudesmol (1.74%), trans-Geraniol (1.60%), α-Amorphene (1.52%), and γ-Cadinene (1.04%).

Larvicidal Activity of Essential Oil and Pyrethroid

The larvicidal effectiveness of essential oil and pyrethroid across different concentrations is presented in Tables 2 and 3. The essential oil was diluted to concentrations of 0.3125, 0.625, 1.25, 2.5, 5, 10, and 20 mg/ml, while pyrethroid was prepared at 3.125, 6.25, 12.5, 25, 50, and 100 µg/ml. The larvicidal activity of both substances was concentration-dependent, with higher concentrations exhibiting stronger efficacy. Specifically, for *Melaleuca cajuputi* essential oil, the larvicidal effect increased from 15.96±2.69% at 0.3125 mg/mL to 100.00% at 20 mg/ml. Similarly, pyrethroid demonstrated an increase in larvicidal efficacy from 42.60±2.41% at 3.125 µg/mL to 100.00% at 100 µg/ml.

Table 2: Effects of Pyrethroid on *Rhipicephalus sanguineus* larvae.

Concentration of Pyrethroid (µg/ml)	Larval mortality (%)
100	100.00±0.00
50	89.76±3.48
25	77.76±2.35
12.5	66.93±2.00
6.25	54.74±2.48
3.125	42.60±2.41
Positive control (pyrethroid 750 µg/ml)	100.00±0.00
Negative control (distilled water)	2.25±0.15

Table 3: Effects of *Melaleuca cajuputi* essential oils on *Rhipicephalus sanguineus* larvae.

Concentration of essential oils (mg/ml)	Larval mortality(%)
20	100.00±0.00
10	86.50±3.45
5	79.98±1.82
2.5	67.96±5.55
1.25	49.27±12.98
0.625	35.94±5.69
0.3125	15.96±2.69
Positive control (pyrethroid 750 µg/ml)	100.00±0.00
Negative control (ethanol 96%)	2.35±0.25

Table 4: Lethal doses of *Melaleuca cajuputi* essential oils and Pyrethroid against *Rhipicephalus sanguineus* larvae.

Treatments	LD_{50}	95% CL	LD_{90}	95% CL	LD_{99}	95% CL
Melaleuca cajuputi (mg/ml)	1.27^{b}	1.11 -1.44	8.96^{b}	7.39-11.24	43.95^{b}	31.82 -65.06
Pyrethroid (µg/ml)	5.14^{a}	4.36 -5.92	45.17^{a}	37.84-55.84	265.73^{a}	189.93 -403.00

LD50, LD90, and LD99 refer to the lethal doses required to kill 50%, 90%, and 99% of the population, respectively. CL represents the 95% confidence interval. Superscript letters (a or b) within the same column indicate statistically significant differences among treatments (P < 0.05).

Table 4 presents the lethal doses (LD50, LD50, and LD99) for *Melaleuca cajuputi* essential oil and pyrethroid. *Melaleuca cajuputi* had the highest LD50 (1.27 mg/ml), while pyrethroid had the lowest (5.14 µg/ml). Similarly, pyrethroid required the lowest doses for LD_{90} and LD_{99}. Statistical analysis revealed significant differences in LD50, LD90, and LD99 values between pyrethroid and the essential oils (P<0.05). While less potent than pyrethroid, *Melaleuca cajuputi* essential oil remains a promising natural alternative for tick control in dogs.

Adulticidal Activity of Essential Oil and Pyrethroid

In this study, the lethal dose required to kill 50% of larvae (LD50) of *Melaleuca cajuputi* essential oil, as determined from the larval bioassay, was used to assess its impact on engorged female ticks. As presented in Table 5, the egg production index for *Melaleuca cajuputi* was 62.91±1.49%, whereas for pyrethroid, it was significantly lower at 0.49%. Additionally, pyrethroid reduced egg-laying capacity by 99.38±0.77%, while *Melaleuca cajuputi* essential oil resulted in a 28.27±3.47% reduction. The egg hatchability rate was 85.73±2.07% for the 96% ethanol control and 48.99±1.11% for *Melaleuca cajuputi* essential oil. These findings suggest that *Melaleuca cajuputi* essential oil effectively reduces egg production, achieving an inhibition rate of 42.82±2.21% at the LD50 concentration used in the larval assay. This reduction is within the range of reproductive inhibition reported for other natural acaricides, underscoring the potential of *Melaleuca cajuputi* essential oil to disrupt tick population dynamics.

Table 5: The effects of *Melaleuca cajuputi* essential oils on engorged females *Rhipicephalus sanguineus* ticks.

Treatments	EPI	RO (%)	EH (%)	EP (%)
Ethanol 96%	87.80 ±2.30[a]	-	85.73 ±2.07[a]	-
Melaleuca cajuputi	62.91 ±1.49[b]	28.27 ±3.47[b]	48.99 ±1.11[b]	42.82 ±2.21[b]
Pyrethroid	0.49± 0.06[c]	99.38 ±0.77[a]	00.00[c]	100.00 ±0.00[a]

EPI: egg production index; **RO:** reduction in oviposition; **EH:** eggs hatched; **EP:** efficiency of production. Superscript letters (a, b, or c) within the same column denote significant differences among treatments ($P< 0.05$).

DISCUSSION

Ticks infesting dogs have shown resistance to ectoparasiticides in multiple regions, posing challenges for effective tick control and management (Becker *et al*., 2019; Higa *et al*., 2020). Plant essential oils effectively kill tick larvae and reduce adult tick reproduction, offering a natural alternative to synthetic parasiticides (Adenubi *et al*., 2018). This study assessed the effectiveness of *Melaleuca cajuputi* essential oil in controlling tick infestations by targeting both larvae and engorged female ticks. The results demonstrated that the essential oil not only successfully eliminated tick larvae but also significantly reduced the reproductive capacity of adult female ticks, highlighting its potential as a natural alternative for tick management. The observed larvicidal effects and reduction in reproductive ability may be attributed to the bioactive components of the essential oil, which either exhibit larvicidal properties individually or act synergistically to enhance larvicidal activity and suppress tick reproduction. The essential oils are abundant in α-Terpineol, a compound that has been proven highly effective in eliminating *Rhipicephalus appendiculatus* ticks (Lwande *et al*., 1998) and *Ixodes ricinus* nymphs (Jaenson *et al*., 2005). Additionally, β-Linalool, present in these essential oils, has been found to inhibit acetylcholinesterase activity in ticks, disrupting their nervous system function and leading to mortality (Alimi *et al*., 2022). Another significant component, Eucalyptol (1,8-Cineole), has demonstrated potent acaricidal activity, exhibiting both larvicidal and adulticidal effects against *Rhipicephalus (Boophilus) microplus* (Castro *et al*., 2018; Adenubi *et al*., 2021). Moreover, Guaiol, a key constituent of the essential oil, has been shown to effectively eliminate *Rhipicephalus* species ticks, even those that have developed resistance to commonly used acaricides (Luns *et al*., 2021). Furthermore, D-Limonene has been reported to be lethal to both larvae and adult *Rhipicephalus microplus* ticks, confirming its strong acaricidal potential (Peixoto *et al*., 2015). Similarly, Eugenol has exhibited significant larvicidal and adulticidal activity against *Rhipicephalus sanguineus*, a tick species that infests dogs, reinforcing its effectiveness as a natural tick control agent (Oliveira *et al*., 2022; Silva *et al*., 2023).

The larvicidal properties of this essential oil, along with its ability to reduce the reproductive capacity of engorged female ticks, may be attributed to the direct action of its bioactive compounds. When essential oils come into contact with larvae and adult ticks, they initially interact with the cuticle, the outer protective layer. Upon penetration, the active components diffuse into the hemolymph, where they can reach and disrupt vital internal organs (Remedio *et al*., 2015). Additionally, some compounds within the essential oil may specifically target the salivary glands, potentially altering the concentration of blood components and interfering with the efficient absorption of nutrients necessary for survival and reproduction. Research has established a strong connection between the digestive and reproductive systems of ticks, suggesting that any disruption to digestion can directly impair reproductive success (Remedio *et al*., 2016).

The reproductive system of ticks, particularly the ovaries, plays a crucial role in egg development. The ovaries consist of an epithelial layer surrounding oocytes at various developmental stages (Oliveira *et al*., 2005). Damage to these oocytes caused by exposure to essential oils can severely affect the female tick's ability to produce viable eggs. The active compounds in the essential oils may alter oocyte morphology, leading to structural changes such as vacuole formation within the germinal vesicles and cytoplasm, fusion or degradation of yolk granules, and, in severe cases, oocyte rupture (Monteiro *et al*., 2021; Silva *et al*., 2023). These disruptions ultimately reduce the reproductive success of ticks, highlighting the potential of essential oils as a natural alternative for tick control.

Moreover, essential oils interfere with the transfer of lipids from adipose tissue to the ovaries in ticks, leading to a reduction in cholesterol levels within the developing eggs. This decline in cholesterol content compromises egg viability, ultimately reducing the reproductive success of female ticks (Oliveira *et al*., 2022). The ability of essential oils to exhibit both larvicidal activity and detrimental effects on engorged female ticks may also be linked to their impact on the tick nervous system. By disrupting neural function, these oils can interfere with nerve impulse transmission, leading to impaired movement and feeding mechanisms. Several studies have documented that certain plant-derived essential oils induce both neurological and structural alterations in ticks (Castro *et al*., 2018). Some bioactive compounds found in essential oils act as acetylcholinesterase inhibitors, which disrupt neural signal transmission and trigger neurotoxic effects that compromise tick survival (Higa *et al*., 2020). In this study, β-Linalool and Eucalyptol, which were identified as major constituents of the examined essential oil, were found to inhibit acetylcholinesterase activity (Alimi *et al*., 2022). This suggests that these compounds play a critical role in the essential oil's efficacy against both larvae and adult female ticks. The observed neurotoxic effects further highlight the potential of essential oils as a natural alternative for tick control in dogs. To fully understand the impact of these essential oils on tick populations, further research is needed. Future studies should focus on investigating structural changes in the reproductive organs of female ticks and their eggs following exposure to essential oils, which could provide a strong scientific foundation for explaining their lethal effects on larvae and their influence on the reproductive potential of gravid females. Furthermore, exploring the interactions among the bioactive compounds present in the essential oil whether synergistic, additive, or inhibitory will be crucial in optimizing its efficacy. Understanding these interactions is essential for the practical application of this essential oil and may contribute to the identification of other plant-derived essential oils with similar chemical properties for use in controlling tick infestations in dogs.

The observed 42.82 ± 2.21% reduction in egg production at the LD_{50} concentration of *Melaleuca cajuputi* essential oil indicates a biologically significant impact on tick reproductive capacity. Although this concentration was initially determined based on larval mortality, its additional effect on adult reproduction suggests that even sublethal doses can impair population sustainability. A nearly 43% inhibition in egg production could substantially slow population growth over multiple generations, especially when integrated with other control strategies. Compared to some other natural acaricides, this level of reproductive inhibition is promising, highlighting the potential of *Melaleuca cajuputi* essential oil as a dual-action agent affecting both survival and reproduction. Previous *in vivo* studies have demonstrated the potential of essential oils as effective acaricidal agents when applied topically. For instance, Nirbhay *et al*. (2018) reported successful reduction of tick infestations using essential oil-based formulations on cattle, highlighting their potential for practical application in veterinary settings. Incorporating similar in vivo models will be critical for evaluating the real-world efficacy and safety of *Melaleuca cajuputi* essential oil.

Despite the promising acaricidal activity of *Melaleuca cajuputi* essential oil observed in this study, certain limitations should be acknowledged. The findings are based on in vitro assays, and further in vivo studies are necessary to evaluate the oil's efficacy and safety under practical conditions. Additionally, the composition of essential oils can vary depending on factors such as plant origin, harvest time, and extraction techniques, potentially affecting their consistency and biological activity. The present study also did not assess morphological changes in tick larvae after exposure, which could provide further insight into the essential oil's mechanism of action. Future research should aim to evaluate the long-term effects of *Melaleuca cajuputi* essential oil, its safety profile for use in dogs, and its potential to cause skin irritation or systemic toxicity. Standardization of essential oil composition and studies on the stability and shelf-life of the product are also important to ensure consistent efficacy. Moreover, morphological and physiological evaluations of treated ticks could help elucidate the specific mode of action of the oil's bioactive components.

CONCLUSIONS AND RECOMMENDATIONS

Melaleuca cajuputi essential oil exhibits significant *in vitro* larvicidal activity against *Rhipicephalus sanguineus*, with a median lethal dose (LD_{50}) of 1.27 mg/ml, and markedly reduces tick reproductive capacity. These findings suggest that *Melaleuca cajuputi* essential oil shows promise as a natural acaricide, pending validation through *in vivo* studies. Further research is needed to elucidate its mode of action and to evaluate potential synergistic or antagonistic interactions among its bioactive constituents. Future trials should test topical application on tick-infested dogs to assess both acaricidal efficacy and dermal safety under real-world conditions. The present results support the potential of *Melaleuca cajuputi* essential oil as a natural alternative to synthetic antiparasitic agents, which are increasingly challenged by resistance in tick populations.

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NOVELTY STATEMENT

This study presents an innovative strategy for tick control by utilizing essential oil derived from *Melaleuca cajuputi* leaves to target both larvae and adult female ticks. The findings reveal that this essential oil effectively eliminates tick larvae and significantly reduces the reproductive capacity of gravid *Rhipicephalus sanguineus* females. These results highlight its promising application as a natural alternative for tick prevention and treatment in dogs. Further research, including field trials and toxicity assessments, is necessary to optimize its formulation and ensure its safety and efficacy for widespread use.

AUTHOR'S CONTRIBUTIONS

Nguyen Van Vui was responsible for conceptualizing and designing the experiments. Nguyen Thi Kim Quyen and Nguyen Van Vui conducted the experimental work. Nguyen Van Vui performed the data analysis and prepared the initial manuscript draft. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest

Authors declared no conflict of interest.

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