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Article

Babaçu (*Attalea Speciosa*) Residue Bio-Oil Repels Livestock Tick Larvae *Rhipicephalus (Boophilis) Microplus*

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Abstract: *Rhipicephalus* (*Boophilis*) *microplus* (also known as southern cattle tick or Asian blue tick) is one of the most detrimental and prolific tropical cattle parasites. Currently, chemical acaricides used against these ticks have been less effective due to increased pesticide resistance stemming from overuse of these treatments. We propose a novel tick repellent to address the waning efficacy of chemical treatments for *R.* (*B.*) *microplus* on cattle. In the search for an alternative, seven concentrations (100%, 50%, 25%, 12.5%, 6.25%, 3.13%, and 1.57%) of babaçu (*Attalea speciosa*) residue bio-oil were produced by hydrothermal pyrolysis at 180 °C. The repellency of these bio-oil concentrations was assessed using a tick climbing test. Additionally, toxicity tests were performed by organic chemical analysis and polyaromatic hydrocarbon analysis. The repellency to *R.* (*B.*) *microplus* tick larvae was 100% for concentrations higher than 3.13% babaçu residue bio-oil concentration. However, the 1.57% concentration can be promising even with less repellent effects (though still being 93.7% effective) due to lower toxicity. This is an innovative approach for overcoming drug resistance in these ticks. Future research can test other bio-oils and pyrolysis products as tick repellents and botanical acaricides to further diversify options for better managing these parasites in Brazil and elsewhere in the tropics.

Keywords: *Attalea speciosa*; bio-oil; cattle; larvae; livestock; pyrolysis; repellant; *Rhipicephalus (Boophilis) microplus*; tick; toxicity

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1. Introduction

Rhipicephalus (*Boophilus*) *microplus* is a monoxenous cattle tick found in tropical and subtropical regions of the world [1]. This particular ixodid tick has a wide global distribution, including Central and South America, Asia [1], and Africa [1–3]. This species originated in India and spread throughout the world through animal trade [4]. While *R.* (*B.*) *microplus* has been mostly eradicated in the USA [1], these cattle pests are problematic not just in sub-tropical and tropical regions but also on islands in Polynesia, the Caribbean, and even larger island nations such as Madagascar [1]. Its occurrence has been impacting Brazil's national cattle herd for decades, with annual losses of around USD 3.2 to 3.9 billion [5]. Losses include lower meat yields from beef cattle as well as 50% lower milk production from dairy cattle [6]. Moreover, the infestation of *R.* (*B.*) *microplus* on beef and dairy herds alters their leather quality, and it is the primary vector responsible for outbreaks of tick-borne diseases such as anaplasmosis and babesiosis [7].

Tropical regions in South America such as Brazil's Amazon have had a rapid recent expansion in beef cattle herds and stocking densities [8], where *R.* (*B.*) *microplus* can sequentially have up to six generations per year [9] due to their three-to-four-week life cycle [1]. Higher cattle stocking densities increase the likelihood of the transfer of these ticks between animals [10]. In addition to cattle and other domesticated livestock, these ticks can host on wild animals such as deer [1] and can also survive during their larval stage for three to four months without feeding, making these parasitic pests ever pervasive.

Cultural controls such as pasture rotation are not practical for most cattle producers since livestock have to be absent for six to nine months [1]. In general, control is based on the application of topical and systemic acaricides after identifying adult ticks by spraying, dipping, pouring, and/or injecting [11]. Based on this scenario, the number of doses tends to be high, varying with the method of application, time of year, animal breed, and cost of treatment [12]. The efficacy of backpack sprayers, which are predominantly used in Brazil, is lower than power sprayers and spray races [11]. In addition, the control of *R.* (*B.*) *microplus* in Brazil is further challenged by incorrect acaricide dosage as well as the improper management of spray races and dipping vats [13].

The genomic diversity of *R.* (*B.*) *microplus* has been attributed to increasing reproductive capacity, the ability to transmit pathogens, and general adaptability [4] to changing environmental conditions (e.g., resistance to acaricides) [14]. Over the years, acaricides have shown limitations due to their overuse, with the emergence of *R.* (*B.*) *microplus* resistance to six out of seven major classes of chemical acaricides currently marketed in Brazil. *R.* (*B.*) *microplus* has shown resistance to acaricides starting with organophosphates [13]. Organophosphate resistance for this tick has been documented in the Brazilian states of Bahia [15], São Paulo [16], and Mato Grosso do Sul [17,18]. Resistance next developed in Brazil for formamidines (e.g., amitraz) [13,16] followed by pyrethroids [13,17,18] and macrocyclic lactones [13]. Acaricides introduced more recently over the past 30 years in Brazil have had similar resistance issues such as phenylpyrazoles (e.g., fipronil) [19] and benzoylphenylureas [13]. Resistance has been variable by region and by acaricide. For example, in the Bahia state in northeastern Brazil, there are high efficacies for fipronil (97.18%) and fluazuron (100%) compared to amitraz (32.35%), organophosphates (23.18%), and pyrethroids (11.9%) [20]. Resistance has not been reported yet to isoxazolines, which were introduced to Brazil in 2022 [13]. *R.* (*B.*) *microplus* is also resistant to chemical acaricides in India [21] and Mexico [22,23]. In Mexico, *R.* (*B.*) *microplus* is also resistant to ivermectin [22,24].

Based on the global economic importance of *R.* (*B.*) *microplus* and the difficulty in controlling it, there is a clear need to develop alternatives to combat its parasitism such as acaricides derived from botanicals, pathogens, and vaccines. Botanical acaricides can kill, interfere with reproduction, and/or repel *R.* (*B.*) *microplus* [25]. In their global review, Pavela et al. (2016) reviewed 238 plant species traditionally used against ticks [26]. Borges et al. (2011) highlighted 55 plant species belonging to 26 plant families that have been tested against *R.* (*B.*) *microplus* in particular [27]. Numerous studies have evaluated the potential for herbal preparations and non-metallic, non-toxic nanoparticles produced from natural ingredients, botanical nanoformulations, metal nanoparticles [28], as well as botanical controls [28–32].

Alcalá-Gomez et al. (2024) showed that isolated specialized strains of the pathogenic fungus *Beauveria bassiana* caused *R.* (*B.*) *microplus* to have 96% and 100% mortality in the lab [33]. Similarly to acaricides, the genomic diversity of *R.* (*B.*) *microplus* also contributes to its adaptability to vaccines. However, there have been promising developments of both traditional and recombinant vaccine antigens [34,35].

Another mode of action of botanicals against *R.* (*B.*) *microplus* is to act as a tick repellent. Repellent use should be considered, since it can disrupt the parasitic cycle of *R.* (*B.*) *microplus*, thus mitigating its effects on cattle health and subsequent economic impacts. Repellents are chemical substances that cause a tick to move away from its source, while deterrents are chemical substances that inhibit feeding in situations where it would typically take place [36]. Although some commercial repellent products such as N, N-diethyl-m-toluamide, popularly known as DEET, are highly efficient, their repellency periods are relatively short, around 4.8 h or less depending on the concentration [37]. This makes the use of DEET infeasible under field conditions, highlighting the need for new approaches. Essential oils (EOs) and products derived from essential oils, also known as essential oil compounds (EOCs), can be used to repel and control ticks. Gonzaga et al. (2023) reviewed 31 journal articles on EOs and EOCs used for tick control with over half conducted in Brazil, 70% on cattle, and almost half on *R.* (*B.*) *microplus* in particular [38]. However, none of these reviews on botanicals and essential oils have evaluated compounds (e.g., bio-oils) produced during pyrolysis.

The composition of bio-oils from pyrolysis includes a range of organic compounds. Bio-oil (BO) has a strong odor and low pH (2.5) [39,40]. This can make BO an effective repellent. BO is immiscible in water [41], favoring the viability of its repellency even in regions with high rainfall, such as the Amazon. However, BO properties can vary depending on biomass and the type and conditions of pyrolysis, such as heating rates and reaction temperature [41–43]. This can potentially affect its chemical composition and repellency capacity. Furthermore, some toxicological studies suggest BO use can pose risks to human health [44–46]. Any hazards must be considered for animal use as a tick repellent.

Producing BO from waste can mitigate environmental problems and add value to the crop residues that would normally be discarded. The babaçu palm (*Attalea speciosa*) is considered one of the more important agricultural products in northeastern Brazil. Currently, the production of Brazilian babaçu almonds is 54,300 metric tons per year [47]. For each metric ton (1000 kg) of babaçu processed, around 922 kg (92.2%) is wasted [48,49], generating about 120,000 $\times$ 92.2% = 110,640 metric tons of waste per year, usually disposed directly into the environment [49]. Based on its organic composition and biomass availability, we hypothesize that babaçu residue BO has technical viability as an *R.* (*B.*) *microplus* repellent, given its low viscosity as well as chemical and sensory features. Therefore, the goal of this research is to determine if bio-oil pyrolyzed from babaçu waste is an effective repellent for *R.* (*B.*) *microplus* resistance ticks. Our specific objectives were to (1) identify the specific compounds in our pyrolyzed babaçu bio-oil that may be responsible for repelling these ticks and (2) determine how much babaçu bio-oil repels *R.* (*B.*) *microplus* ticks in vitro in a laboratory study.

2. Results and Discussion

2.1. Babaçu Bio-Oil Chemical Composition

Babaçu bio-oil (BO) has many interesting chemical components with repellence potential (Table 1). Phenols with a OH group and OCH_3 seem to have substantial potential in repelling the brown ear tick (*Rhipicephalus appendiculatus*) [50]. For example, crude repellent blends from the cattle perineal region has been shown to be a natural tick repellent [51]. The major constituents of the odor were characterized and analyzed using climbing assays [52]. The most active compound was found to be 4-methyl guaiacol, which repelled the savannah tsetse fly or *Glossina pallidipes* (Diptera: Glossinidae) [53] and the brown ear tick [50]. Kariuki et al. (2019) also tested the repellency of ten structural analogs of 4-methyl guaiacol from babaçu BO, including guaiacol, 4-methyl guaiacol, 4-ethyl guaiacol, eugenol, and 4-allyl-2,6-dimethoxyphenol (Table 1). These five compounds exhibited a high degree of repellency [50].

Table 1. Chemical compounds of babaçu residue bio-oil analyzed by CG-MS.

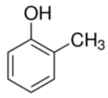
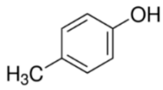
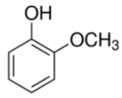
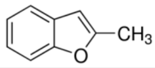
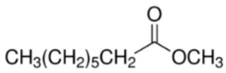
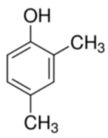
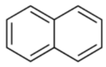
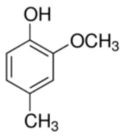
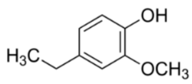
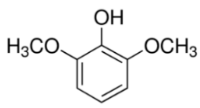
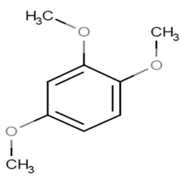
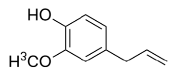
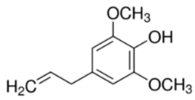
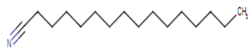
Compound	Area (%)	Formula	Chemical Characteristics
2-Methylphenol (o-Cresol)	2.10		Toxic if swallowed or in contact with skin, causes skin burns and eye damage.
4-Methylphenol (p-Creosol)	2.80		Toxic if swallowed or in contact with skin, causes skin burns and eye damage.
2-Methoxyphenol (Guaiacol)	3.60		Aromatic oil is used medicinally as an expectorant, antiseptic, and local anesthetic. Tick repellent.
Undecane	1.32	$CH_3(CH_2)_9CH_3$	Insoluble in water and used to make other chemicals
2-Methylbenzofuran	0.78		Strength odor, recommend smelling in a 0.10% solution or less.
Methyl octanone	0.85		May irritate eyes, skin, and respiratory tract.
2,4-Dimethylphenol	1.24		Food additives as flavoring agents, adhesive, antimicrobial. Tick repellent.
Naphthalene	0.90		Solid polycyclic hydrocarbon, irritating, strong mothball odor, used as moth repellents.
2-methoxy-4-methyl phenol or 4-methyl guaiacol (creosol)	3.00		Most active compound from crude odor collected from cattle perineal region, also found to be repellent to savannah tsetse and tick.
4-Ethylguaiacol	2.69		Tick repellent.
Tridecane	0.92	$CH_3(CH_2)_{11}CH_3$	Repeated or prolonged skin contact may irritate or redden skin, progressing to dermatitis. One of the major chemicals secreted by some insects to defend against predators.
2,6-Dimethoxyphenol	2.72		
1.2.4-Trimethoxybenzene	2.12		

Table 1. *Cont.*

Compound	Area (%)	Formula	Chemical Characteristics
Eugenol or 4 allylguacol or 2-methoxy-4-propenylphenol	0.98		Spicy odor; the taste of clove is used in perfumeries, flavorings, and in medicine (local antiseptic and analgesic).
Dodecanenitrile	2.59	$CH_3(CH_2)_9CH_2CN$	
Pentadecane	1.15	$CH_3(CH_2)_{13}CH_3$	
Heptadecane	1.00	$CH_3(CH_2)_{15}CH_3$	Component of essential oils from plants; also, has a role as a plant metabolite and a volatile oil component.
4-Allyl-2,6-dimethoxyphenol (Methoxyeugenol)	1.02		Tick repellent.
Palmitonitrile	0.92		
Fatty acids and methyl esters	53.9		Found naturally in various plant and animal fats and oils, although slightly irritating to mucous membranes, has very low toxicity, and used in many soaps and shampoos.

Source: the majority and main chemical usefulness of the organic compounds found by GC-MS were investigated by the CAS SciFinder® [54], ChemSpider [55], ACToR [56], PubChem [57], TGSC Information System [58], and ECHA [59] databases.

Due to its significant number of chemical compounds (Table 1), babaçu residue BO may have no single specific mechanism of action but rather multiple ones. Babaçu residue BO has compounds that may cause different modes of action by producing various biological changes in target organisms. Some of these effects may be cytotoxic (Table 1), mutagenic, and carcinogenic (Table 2). BO chemical characteristics are dependent on the organic source material and the thermal treatment processes used, including temperature, retention time, and particle sizes or granulometry [60]. Bio-oils can vary significantly in their composition, since they contain different constituents at varying concentrations. These multicomponent mixtures are derived primarily from depolymerization and fragmentation reactions of lignocellulose, cellulose, hemicellulose, and lignin [61].

Table 2. The concentration of individual and total poly-aromatic hydrocarbon in babaçu residue bio-oil.

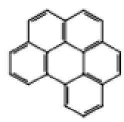
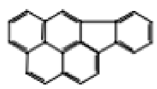
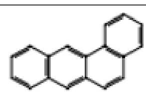
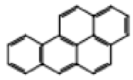
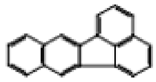
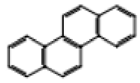
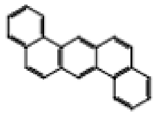
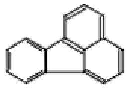
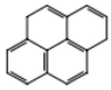
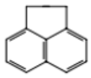
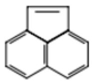
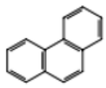
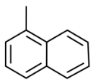
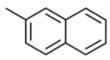
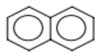
Effect	PAH (Nomenclatura—IUPAC)	Structure	Concentration (ηg mL^{-1})
Carcinogenic	Benzo (g,h,i) perylene		42.09
	Indeno (1,2,3-cd) pyrene		39.51
Carcinogenic and Mutagenic	Benzo (a) anthracene		127.05

Table 2. *Cont.*

Effect	PAH (Nomenclatura—IUPAC)	Structure	Concentration (ηg mL^{-1})
	Benzo (a) pyrene		70.91
	Benzo (b) fluoranthene		58.32
	Benzo (k) fluoranthene		58.30
	Chrysene		123.18
	Dibenz (a,h) anthracene		56.98
	Fluoranthene		240.62
	Pyrene		239.37
Mutagenic	Acenaphthene		415.93
	Acenaphthylene		300.63
	Anthracene		271.92
	Fluorene		333.66
Mutagenic and Toxic	Phenanthrene		274.1
Toxic	1-Methylnaphthalene		435.65
	2-Methylnaphthalene		458.58
	Naphthalene		384.55
	TOTAL		3931.35

Source: the majority and main chemical usefulness of the organic compounds found by GC-MS were investigated by the CAS SciFinder® [54], ChemSpider [55], ACToR [56], PubChem [57], TGSC Information System [58], and ECHA [59] databases.

The guaiacols are formed from the lignin fraction, whereas the miscellaneous oxygenates, sugars, and furans form the cellulose and hemicellulose biomass fraction. The esters, acids, alcohols, ketones, and aldehydes are likely derived from the decomposition of miscellaneous oxygenates, sugars, and furans. The fatty acids and their methyl esters make up the most significant fraction of compounds found in babaçu residue BO, which explains their physical and chemical properties such as aspect and washability potential. Among the compounds found in this fraction, lauric acid and its corresponding ester, methyl laurate, represent more than half of the fatty acids and methyl esters found in BO. Myristic acid and its methyl ester made up 8% of the total and had essential applications due to its saturated chain of fourteen carbons being used in pharmaceutical and cosmetic preparations [62].

In general, fatty acids are the most effective antimicrobial substituents in human skin fat [63]. Takigawa et al. (2005) reported that specified fatty acids found in high concentrations in human skin are lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0). These three acids and their methyl ester have been found in BO in concentrations of 24.35%, 7.51%, 5.86%, 2.95%, 2.23%, and 1.79% [64].

The polyaromatic hydrocarbons evaluated are considered by the United States Environmental Protection Agency as priority pollutants given their toxic potential, the majority being carcinogenic and mutagenic. These compounds ranged from 39.51 to 458.58 ηg mL^{-1}, adding up to 3931.35 ηg mL^{-1} (Table 2). The characteristics of these contaminants warrant their inclusion in most environmental and human health monitoring programs [65,66].

Contamination by polyaromatic hydrocarbons (PAHs) should be evaluated once BO is used as a repellent for ticks on cattle. Since BO repellent is spread on the underside and legs of cattle, it can contaminate them through the skin and accidental ingestion. From a food security perspective, PAHs can contaminate humans through consuming meat, milk, and dairy products, and because they are fat-soluble compounds, PAHs are easily absorbed [67]. PAHs can also be biotransformed by the action of specific enzymes, often present in large vertebrates such as mammals and birds [68]. This biotransformation can possibly activate and form carcinogenic agents [69].

Another critical point to be considered is that PAHs are present in foods in general, which are mainly contaminated through food preparation such as smoking and grilling in addition to contamination by packaging and chemical additives [70–72]. Some authors have demonstrated contamination by these compounds, such as 8800 ηg mL^{-1} in black tea (leaves), 566 ηg mL^{-1} in green tea [73], 13.46 ηg mL^{-1} in meat and derivatives [74], and 16.76 ηg mL^{-1} in milk [75]. Additionally, PAHs are also present in the environment such as in water, soil, particulate matter, plants, organisms, and sediments [76–81]. The concentration of PAHs in meat, milk, soil, and plants after applying the bio-oil repellent must be compared to what already exists.

It is worth mentioning that several factors, such as biomass and temperature, affect the distribution of PAHs formed in the BO, a formation that can occur over a wide temperature range. For example, at low temperatures, the distribution of the compound is governed by thermal stability and more stable isomers are formed. At high temperatures, higher-enthalpy PAHs can be generated [82]. That means the biomass burning parameters for bio-oil production can be adjusted to decrease the concentration of PAHs formed, improving the toxicological characteristics of BO used as a repellent.

2.2. Babaçu Residue Bio-Oil Repels Tick Larvae

The repellency to *R.* (*B.*) *microplus* larvae was effective for all concentrations of the climbing assay. Bio-oil (BO) concentrations higher than 3.13% were 99.63% to 100% effective in repelling ticks, while BO concentrations of 1.57% and 3.13% repelled 93.7% and 96.81% of ticks, respectively (Table 3). The tick larvae followed the expected behavior of climbing the paper strips, stopping before reaching the treated BO zone regardless of the BO concentration (Table 3; Figure 1).

Table 3. *Rhipicephalus* (*Boophilis*) *microplus* control, active, and bio-oil treated larvae average and standard deviation, as well as percent repellency for babaçu (*Attalea speciosa*) residue bio-oil climbing test.

Babaçu Residue Bio-Oil (BO)	Active Larvae		Control (No Bio-Oil)			Bio-Oil Treated		
Concentration (%)	Larvae Average	Larvae Std. Dev.	Larvae Average	Larvae Std. Dev.	Repellency %	Larvae Average	Larvae Std.Dev.	Repellency %
1.57	13.6	3.3	12.8	3.5	6.30	0.8	0.9	93.70
3.13	11.0	3.4	10.6	3.3	3.19	0.4	0.8	96.81
6.25	22.1	3.3	22.1	3.3	0	0	0	100
12.5	26.7	5.8	26.7	5.8	0	0	0	100
25	16.6	4.7	16.6	4.7	0	0	0	100
50	13.9	4.8	13.9	4.7	0.37	0	0	99.63
100	24.6	5.1	24.6	5.1	0	0	0	100

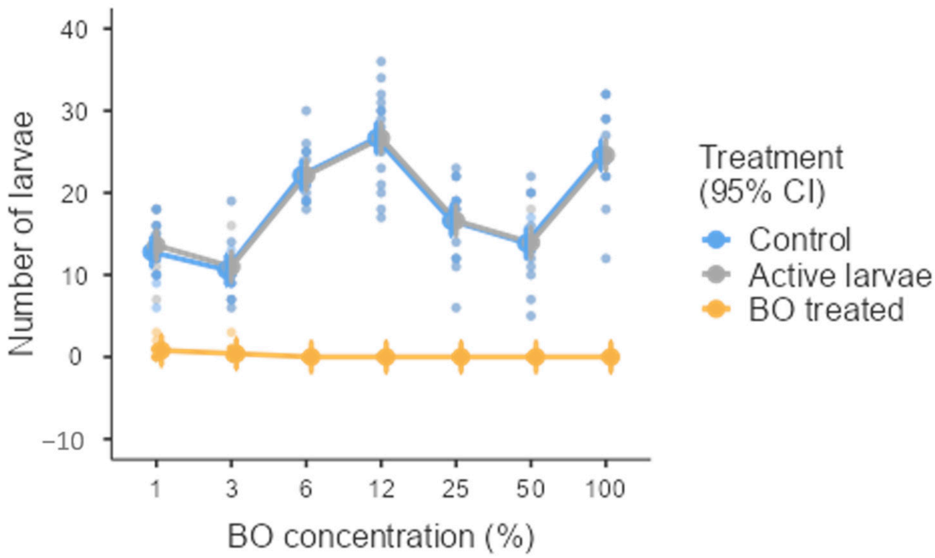


Figure 1. Percentage of *Rhipicephalus* (*Boophilis*) *microplus* larvae repelled by babaçu (*Attalea speciosa*) residue bio-oil applied in concentrations ranging from 1.57% to 100%. The circles are the means of the number of larvae responding to the climbing test, while the bars are the confidence interval (95%) of the mean, and the blue, gray, and orange dots are the number of larvae repetitions measured for each treatment.

These results suggest that the 1.57% BO concentration can be more promising due to cytotoxicity in the bloody cells of fishes (*Astyanax lacustris*), even though it did not show the same behavior in vegetable cells (*Allium cepa*) [83]. Also, skin sensitization and allergies must be considered with high concentrations of BO due to its chemical composition (Table 1). In addition, smaller concentrations can result in both repelling ticks and may be more economically viable. In this context, field tests should be considered with concentrations of around 1.57%.

Babaçu residue bio-oil was effective in repelling ticks in vitro. This may theoretically translate to preventing tick larvae attachment on its favorite host (e.g., cattle) and disturbing the life cycle of the cattle tick at the farm level. This is a plausible hypothesis, but many interactions can alter the excellent results of the lab conditions. Dautel (2004) reinforced that concern, considering that an overestimation of repellency in the absence of a host is possible [84]. Moreover, the wide range of methods employed when testing tick repellents makes comparing our results with previous studies more challenging. Studies differ in the timeframe in which repellence is examined, the species and life stages used, the formulation, and the amount of active ingredients tested. Frequently, 100% concentration corresponded to the gross extract, where the exact amounts of extracted substances are unknown [85]. Taken together, these issues highlight the importance of developing a standardized protocol for testing tick repellents.

Regardless of these limitations, several plants and essential oils from plants also exhibit repellent properties against hematophagous arthropods, including ticks [86]. Since *R.* (*B.*) *microplus* is considered an emerging economic and health problem in tropical and subtropical regions [87–89], preventing exposure to tick-infested areas by adopting repellents can be effective in preventing ticks and tick-borne diseases in livestock [86,90]. Indeed, environmentally friendly methods to control tick incidence or even complement the use of chemical treatments are always welcome by farmers and ranchers. Aquino et al. (2008) emphasized the increased interest in biologically based repellents, likely a response to the public perception that synthetic repellents are unsafe [91].

2.3. Babaçu Residue Bio-Oil Compared to Other Tick Repellents and Acaricides

The repellency of babaçu residue bio-oil in our study was comparable to chemical tick repellents such as DEET, shown by Soares et al. (2010) to have at least 90% repellency against Cayenne tick nymphs (*Amblyomma cajennense*) [37]. Another botanical repellent had slightly lower efficacy. Bravo-Ramos et al. (2023) found that white indigo berry (*Randia aculeata*) seed hydroethanolic extract sprayed on *Rhipicephalus* (*Boophilus*) *microplus*-infested calves reduced tick incidence by 87% using a 20% solution [31]. Our babaçu residue bio-oil at a concentration of 1.57% was 85% effective (Table 3) and was less toxic. It is important for any tick repellent or acaricide to not negatively impact cattle. For example, Gonzaga et al. (2023) carried out a review of 31 journal articles on essential oils and essential oil compounds used for tick control and found that 86.6% of botanical controls had no adverse reactions with animal hosts [38]. Future research should test babaçu residue bio-oil for tick mortality in vivo, since botanical acarcide extracts can have variable effectiveness against *R.* (*B.*) *microplus*, particularly in the field versus the laboratory.

Banumathi et al. (2017) reviewed different solvent extracts from 67 plants used against *R.* (*B.*) *microplus* larvae and adults, where mortality on average was higher for in vitro (71.4%) versus in vivo (39.4%) studies as well as tick larvae (83.7%) compared to adults (53.8%) [28]. Borges et al. (2011) reviewed 57 plant species across 26 plant families, finding in vitro efficacies of botanical acaricides, particularly against tick larvae (50% to 100%) and egg laying (11.5% to 79%), with more variable effectiveness against engorged female adult ticks (2.3% to 100%) [27]. Efficacies for in vivo tests were lower, ranging from 27.3% to 92.4%, with greater effectiveness of hexanic and chloroformic extracts versus ethanolic extracts [27]. However, Banumathi et al. (2017) showed greater tick mortality for ethanol (74.3%) and methanol (71.5%) extracts versus other types [28]. Gonzaga et al. (2023) reviewed 31 journal articles on anti-tick botanicals derived from 19 plant species, but their efficacy could not be meaningfully compared due to differences in methods [38].

Botanical products against ticks are promising [92]. While synthetic acaricides have successfully controlled ticks [93], resistance to chemical pesticides has become more problematic [13,94] and accelerated due to *R.* (*B.*) *microplus* having up to six generations per year [95]. Therefore, there is a critical need for integrated approaches for managing ticks [13,96]. Rodriguez-Vivas et al. (2018) discuss both conventional/traditional as well as alternative management strategies to address tick resistance. Conventional/traditional strategies include (1) reducing the frequency of chemical acaricide applications, (2) mixing acarcides for synergistic effects, (3) applying the correct amount of product, (4) removing ticks manually, (5) waiting for host resistance, and (6) releasing sterile male tick hybrids. Agri-environmental management strategies include (1) planting plant species that are not favorable to ticks (e.g., those that trap tick larvae), (2) spelling pasture to disrupt tick life cycles, (3) burning pasture, (4) animal nutrition, (5) vaccination, (6) biological controls (e.g., fungal pathogens such as *Beauveria bassiana*), and (7) the use of plant extracts and essential oils to control ticks [97], as previously discussed.

3. Materials and Methods

3.1. Study Site

The study was carried out at Embrapa Agrosilvopastoral, Sinop, Mato Grosso state, Brazil (11° 51′ S, 55° 35′ W, and 370 m above sea level). We collected 80 engorged females of *Rhipicephalus* (*Boophilus*) *microplus* from our naturally infested herd. The chosen teleogins were kept in Petri dishes at the parasitology lab into a growth chamber (~27 °C; relative humidity of 70%; 12 h photoperiod) until oviposition. After laying, we removed the teleogins to incubate the eggs, adopting the same conditions until the larvae hatched.

3.2. Organic Chemical Compound Analysis

Organic chemical compounds in the babaçu residue bio-oil were analyzed by GC-MS in triplicate in order to be more certain that the bio-oil contained these compounds. We used an Agilent 7890 GC apparatus from Wilmington, Delaware, USA, equipped with a 5975C MS detector and 7683B automatic liquid sampler with a capillary column, HP-5MS 5%-phenyl-95%-dimethylpolysiloxane (30 m, 0.25 mm i.d., film thickness of 0.25 μm) from J&W Scientific, Folsom, California, USA. Oven temperature was programmed to start at 150 °C (1 min), increase to 210 °C at 15 °C minute^{-1} (10 min), and then increase by 5 °C minute^{-1} to 310 °C for 1 min. The injector temperature was set at 270 °C in splitless mode when 1 μL of the sample was injected. The mass selective detector temperatures of the ionization chamber and MS Quad were set to 230 °C and 150 °C, respectively. Electron ionization energy was 70 eV, and the mass range was m/z 30–500 (amu).

Chromatographic peaks from BO samples were used to identify the organic compounds by comparing the experimental m/z values of the compounds from the NIST database. Only compounds with a match above 80% were considered. The relative percentage was calculated using the relationship between the peak area of the compound identified and the sum of peak areas of all majority compounds in the chromatogram. The majority of the main organic compounds found by GC-MS were investigated using the CAS SciFinder® [56], ChemSpider [57], ACToR [58], PubChem [59], TGSC Information System [60], and ECHA [61] databases.

3.3. Polycyclic Aromatic Hydrocarbon Analysis

We also ran polycyclic aromatic hydrocarbon (PAH) analysis in triplicate using a mixture of ISO A and ultrasonic extraction described by Song et al. (2002) [98]. Running the PAH testing in triplicate insured better confidence that the tested bio-oil contained the specific PAH's being tested. Four grams of the sample with 20 mL of toluene was agitated for 30 min at 50 rotations per minute (rpm) on a Kline agitator NT151 from Novatecnica, São Paulo, Brazil. Then, the sample was sonicated for 30 min in an ultrasonic bath at Quimis, São Paulo, Brazil. After the extraction, we centrifuged at 4500 rpm for five minutes on a Sigma 3-16KL centrifuge from Sigma Laborzentrifugen GmbH, Osterode am Harz, Lower Saxony, Germany. The supernatant was collected in a glass tube. The solid residue was extracted twice. All the supernatant was cleaned up on a silica gel column (3 cm) and then eluted with heptane, and the solvent was evaporated under vacuum at 60 °C until 1 mL was reached. Samples were analyzed using the same apparatus used for organic chemical compound analysis (GC-MS). The oven temperature was programmed to start at 50 °C (2 min), increase to 150 °C at a rate of 10 °C minute^{-1}, and then increase to 280 °C at

5 °C $minute^{-1}$, holding it for 9 min. It was then ramped up at 40 °C $minute^{-1}$ to 310 °C, keeping this temperature for eight minutes.

3.4. Climbing Test

The method chosen to test the tick repellence was the climbing test, adapted from Carrol et al. (1989), with larvae 7 to 14 days old [99]. We used filter paper strips measuring 30 × 7 cm, fixing its bottom edge to the Petri dish with double-sided tape. We adopted a spacing of 5 cm above the plate edge for the application of babaçu residue bio-oil (BO), in a band 5 cm high, where 200 µL of the dilution was pipetted. After drying for approximately 15 min, a thin layer of solid glue was set two cm above the BO band for sodium bicarbonate ($NaHCO_3$) adhesion, where the excess was removed manually. At the time of testing, we sprayed the bicarbonate strip with a solution of hydrochloric acid (HCl—0.1 mol) to produce CO_2 to motivate the tick larvae to climb [100].

We evaluated seven successive dilutions primarily prepared in alcohol (97%), 15 repetitions each, and 40 tick larvae per repetition, which was adequate for statistical comparisons. The dilutions of babaçu residue BO were 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, and 1.57%. BO was produced by slow pyrolysis at ~80 °C to 160 °C, using air injection for ~12 h at the AGROBABA Company in Guarulhos, São Paulo state, Brazil (Figure 2).

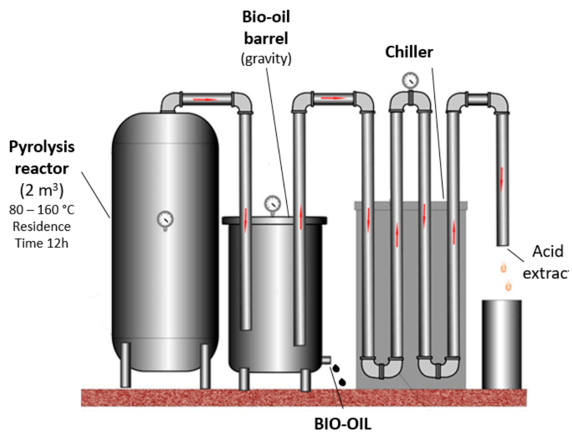


Figure 2. Slow pyrolysis reactor for babaçu residue bio-oil production [101].

Tick larvae were placed in Petri dishes, and larvae behavior was monitored for 10 min uninterrupted. The position of larvae along with the paper strips was then recorded, having as a reference both untreated and the soaked band with BO. Only larvae that moved at least two centimeters (cm) above the paper bottom were considered for analysis. We used control plates for respective dilutions in the same way, with 40 larvae each but with no BO strip. The tick climbing experiment was conducted in a lab at 25 °C.

Active larvae were defined as the sum of larvae that responded to the climbing test, climbing at least 2 cm above the paper bottom, across both control and bio-oil treatments. Tick repellency for BO concentrations was analyzed with general linearized models (GLMs) [102] with Poisson distribution and log as the link function. Differences between climbing tick larvae were tested using a Bonferroni test at 5% significance. The analysis was performed using the statistical software "Jamovi" version 2.2 [103]. The repellency percentage for tick larvae under the BO treatment was calculated as

$$Repellency\ \% = 100\% - ATL\% = 100\% - \frac{ATL_{BO}}{ATL_{Control} + ATL_{BO}} \quad (1)$$

where the active tick larvae (*ATL*) percentage was across both control and BO treatments. The repellency percentage for the control treatment was calculated in a similar way with $ATL_{Control}$ in the numerator instead of ATL_{BO}.

4. Conclusions

A review of prior studies indicated a lower average efficacy of botanical acaricides on adult ticks in vivo (39.4%) versus in vitro (57%) [28]. Therefore, more research needs to be conducted using both in vivo and longer in vitro studies confirming the efficacy of babaçu residue bio-oil (BO) as a tick repellent. In addition, the repellency of babaçu residue BO needs to be evaluated for periods longer than 10 min to determine both the shorter-term efficacy of this repellent as well as any long-term tick tolerance or resistance to this new type of repellent. An improved formulation of the BO may increase its persistence and efficacy. There is also a need to better define the nature of the tradeoff between BO efficacy and safety for cattle found in this study. Future studies should also determine any long-term environmental and health impacts of this BO for both cattle and people. Any toxicity may be addressed in future research by potentially adapting the pyrolysis process to reduce the formation of toxic compounds. Based on our results, we are also proposing a continued investigation of other bio-oils and pyrolysis products as an additional category of tick control. In this case of babaçu residue bio-oil, the pyrolysis feedstock is prevalent, especially in the southern Amazon, northern Cerrado biome, and northeastern Brazil, especially in pastures transitioning back to secondary forest [104].

The use of bio-oil for the control of *Rhipicephalus* (*Boophilus*) *microplus* is an innovative idea to overcome drug resistance to chemical acaricides and repellents. Babaçu palm (*Attalea speciosa*) bio-oil shows significant repellent potential in the climbing test for *R.* (*B.*) *microplus* tick larvae. However, some disadvantages of babaçu residue bio-oil may have to be considered. Babaçu residue bio-oil has the potential for skin sensitization and allergies, and many compounds found in this bio-oil are toxic to animals and humans and are considered to be environmental pollutants. Therefore, more studies have to be performed, focused on the burning parameters for bio-oil production once it can be adjusted to decrease the disadvantages mentioned, primarily in cattle. This can provide another option for repelling *R.* (*B.*) *microplus* on cattle in tropical climates to address the increasing resistance of these ticks to acaricides.

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