

Acaricide activity of *Piper macedoi* Yunck essential oil against *Rhipicephalus sanguineus*

Atividade acaricida do óleo essencial de *Piper macedoi* Yunck contra *Rhipicephalus sanguineus*

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Abstract

This study evaluated the acaricide efficacy of *Piper macedoi* essential oil on larvae of ticks of the species *Rhipicephalus sanguineus*. The essential oil was extracted by hydrodistillation in a Clevenger-type apparatus. The test consisted of six treatments: from the group I to IV, samples corresponded to different concentrations of essential oil (500 $\mu\text{g}\cdot\text{mL}^{-1}$; 250 $\mu\text{g}\cdot\text{mL}^{-1}$; 100 $\mu\text{g}\cdot\text{mL}^{-1}$ and 50 $\mu\text{g}\cdot\text{mL}^{-1}$) diluted in Tween 80 at 2%. Groups V and VI corresponded to the negative controls (with distilled water and Tween 80 to 2%) and the positive control (with acaricide Amitraz at 12.5%), respectively. The essential oil was rich in apiole (39.81%) and dillapiole (26.47%). The essential oil of *P. macedoi* presented an activity against the larvae of *R. sanguineus*, with a better efficiency observed for concentrating 500 $\mu\text{g}\cdot\text{mL}^{-1}$, mortality of 80.67%, indicating a dose-dependent response.

Keywords: Piperaceae; Tick; Larvicidal activity; Apiole; Dillapiole.

Resumo

O objetivo deste estudo foi avaliar a eficácia acaricida do óleo essencial de *Piper macedoi* em larvas de carrapatos da espécie *Rhipicephalus sanguineus*. O óleo essencial foi extraído por hidrodestilação em aparelho do tipo Clevenger. O teste consistiu em seis tratamentos: do grupo I ao IV, as amostras corresponderam às diferentes concentrações de óleo essencial (500 µg.mL⁻¹; 250 µg.mL⁻¹; 100 µg.mL⁻¹ e 50 µg.mL⁻¹) diluído em Tween 80 à 2%. Os grupos V e VI corresponderam aos controles (com água destilada e Tween 80 a 2%) e o controle positivo (com o acaricida Amitraz a 12,5%), respectivamente. O óleo essencial mostrou-se rico em apiol (39,81%) e dilapiol (26,47%). O óleo essencial de *P. macedoi* apresentou uma atividade contra as larvas de *R. sanguineus*, com uma melhor eficiência observada para a concentração de 500 µg.mL⁻¹, mortalidade de 80,67%, indicando resposta dependente da dose.

Palavras-chave: Piperaceae; Carrapatos; Atividade larvicida; Apiol; Dilapiol.

Resumen

El propósito de este estudio fue evaluar la eficacia acaricida del aceite esencial de *Piper macedoi* en larvas de garrapatas de la especie *Rhipicephalus sanguineus*. El aceite esencial se extrajo por hidrodestilación con un aparato tipo Clevenger. El ensayo consta de seis tratamientos: del grupo I al IV, las muestras corresponden a diferentes concentraciones del aceite esencial (500 µg.mL⁻¹; 250 µg.mL⁻¹; 100 µg.mL⁻¹ e 50 µg.mL⁻¹) diluido en Tween 80 al 2%. Los grupos V y VI corresponden a los controles (con agua destilada y Tween 80 a 2%) y el control positivo (con el acaricida Amitraz a 12,5%), respectivamente. El aceite esencial resultó ser rico en apiol (39,81%) y dilapiol (26,47%). El aceite esencial de *P. macedoi* presentó una actividad contra larvas de *R. sanguineus*, con una mejor eficiencia observada para la concentración de 500 µg.mL⁻¹, 80,67% de mortalidad, indicando una respuesta dependiendo de la dosis.

Palabras clave: Piperaceae; Garrapatas; Actividad larvicida; Apiol; Dilapiol.

1. Introduction

Ticks have called the interest of public health and the scientific community due to their impact on the transmission of diseases. These parasites are part of the group of important vectors of infectious agents for animals and the second in importance for humans, only behind the group of mosquitoes (Araújo et al., 2015a). Species such as *Amblyomma cajennense* and *Rhipicephalus sanguineus* are important vectors of *Rickettsia rickettsii*, the etiological agent of spotted fever in Brazil and other Latin American countries (Moraes-Filho, 2009; Vélez et al., 2012).

In Brazil, the two most prevalent species are *Boophilus microplus* and *Rhipicephalus sanguineus*, which mainly affect cattle and dogs, respectively. *R. sanguineus* transmits dog *Babesia canis*, *Babesia gibsonii*, and *Ehrlichia canis*, the unclassified agents of canine hemoplasmosis (haemobartonellosis); *Hepatozoon canis* and *Bartonella vinsonii* (Olivo et al., 2008; Araújo et al., 2015b).

The canine babesiosis, zoonosis transmitted by tick bites whose agents are intraerythrocytic hemoprotozoa of the genus *Babesia*, can also be transmitted to humans (Dantas-Torres & Figueredo, 2006; Araújo et al., 2015b). Another tick-related zoonosis is the Lyme-like infection syndrome (Baggio-Yoshinari disease) caused by bacteria of the genus *Borrelia*, which, although uncommon in Brazil, has confirmed cases in some regions, such as in the States of Tocantins and Espírito Santo (Martins et al., 2009; Mantovani et al., 2007; Yoshinari et al., 2010). The occurrence of *Rhipicephalus sanguineus* in humans is common, especially among people who work in veterinary clinics and kennels (Louly et al., 2006) because dogs from urban or rural areas, raised in kennels, with limited space, are the natural hosts of *R. sanguineus*. An engorged female of this species can make oviposition within human dwellings, leading to the development of adults (Massard & Fonseca, 2004) and, despite the great preference for the dog, *R. sanguineus* can also sting people (Mentz et al., 2016).

Tick control is usually performed with the application of organo-synthetic acaricides indiscriminately, which often results in poisoning of animals and applicators; in acaricidal residues in animal products; development of resistance of ticks to chemical acaricides; as well as environmental pollution (Campos et al., 2012).

An alternative that is increasing in appeal, late, is the search for bioacaricidals of natural source, derived from substances of special plant metabolism that produces a great diversity of compounds with acaricidal action. These resources can be explored using essential oils or compounds as a model for the synthesis of synthetic pesticides (Nwanade et al., 2020).

The use of natural and phyto-therapeutic products show lower toxicity to mammals, slower development of parasitic resistance, and are biodegradable, thus providing low environmental impact and a reduced number of residues in products of animal origin (Chagas, 2004; Veríssimo & Katiki, 2015).

Piperaceae is a plant family possessing a rich diversity in essential oils, that is composed by monoterpenes, sesquiterpenes and arylpropanoids responsible for interesting biological properties (Santos et al., 2001; Oliveira et al., 2013; Salehi et al., 2019). *Piper macedoi* Yunck is a native species of the Brazilian Atlantic Forest, without reports in the literature on pharmacological studies or biological tests. In this context, this evaluate the acaricide efficacy of *P. macedoi* essential oil on *R. sanguineus* larvae. This study is a pioneer for biological assays of *P. macedoi* essential oil.

2. Methodology

2.1 Collection of plant material and chemical analysis of the essential oil

Samples of *Piper macedoi* Yunch were collected in a fragment of the Atlantic Forest located in the Palmeiras Farm, Municipality of Teixeira de Freitas, Bahia, Brazil (17°25'29.4"S 39°41'11.6"W), in February 2016. The species was identified by Dr. Elsie F. Guimarães of the Botanical backyard Research Institute of Rio de Janeiro and deposited in the Herbarium with voucher identification RB 73273. The authorization for collecting species was granted by the Biodiversity Authorization and Information System (SISBIO) (number 31544).

For extracting the essential oil of *P. macedoi*, used in the acaricide assay, fresh leaves (100 g) were submitted to hydrodistillation in a Clevenger-type apparatus for 2 h, in the Interdisciplinary Laboratory of the Federal University of Southern Bahia-UFSB, with a yield of 0.4%. Chemical analysis of the essential oil (solution in dichloromethane at 1mg.mL⁻¹) was performed by gas chromatography coupled to the flame ionization detector (GC-FID) and by GC coupled to mass spectrometry (GC-MS) at the Analytical Platform of Farmaguinhos, FIOCRUZ, Rio de Janeiro. The GC-FID analysis conditions were HP-5MS column (30-m x 0.32 mm i.d. x 0.25 µm film thickness), temperature programming from 60 °C to 240 °C, with an increment of 3 °C.min⁻¹, using hydrogen and synthetic air as carrier gases, with a flow rate at 1.0 mL.min⁻¹. The retention indexes (IR) were determined from the retention time of a homologous series of hydrocarbons (C₈-C₂₈, Sigma-Aldrich Brazil), obtained by GC-FID, under the same conditions of analysis of the essential oils. The column and temperature conditions by GC-MS analysis were the same for GC-FID. Helium (> 99.99%) was used as the carrier gas with a flow rate at 1.0 mL.min⁻¹.

The substances present in the essential oil were identified by comparing their mass spectra with database registration (WILEY7n) and by comparing the Retention Indexes (RI) calculated with those in the literature (Adams, 2017). The quantification of substances in the essential oil was estimated by the chromatogram area obtained using GC-FID.

2.2 *Piper macedoi* essential oil acaricide activity

For the acaricide assay, engorged females of *Rhipicephalus sanguineus* were collected from dogs in veterinary clinics, NGOs and free-living animals from different neighborhoods of the municipality of Teixeira de Freitas.

The females were sanitized with distilled water and dried with sterilized filter paper, then placed in a Petri dish and incubated in an incubator at 25 °C and relative humidity of 85% for oviposition (Labruna et al., 2002). After 14 days, the eggs were collected and packed in a plastic syringe adapted to observe the beginning of hatching and, after that, another 14 days were waited before the acaricide test with the larvae.

The larvae were transferred to a Petri dish containing distilled water and with the aid of a brush. A total of 100 larvae were placed between two pieces of filter paper (2 x 2 cm) impregnated with the treatment solutions (0.4 mL for each repetition). This "sandwich" was placed in a filter paper envelope (6 x 6 cm), sealed by a double tape and stapled (Lambert et

al., 2021). The envelopes were placed in an air-conditioned incubator ($\pm 27\text{ }^{\circ}\text{C}$ and $\text{RH} > 80\%$) and the recording of live and dead larvae was performed after 24 h.

The test consisted of six treatments, from the group I to IV corresponding to the different concentration of *P. macedoi* essential oil (500; 250; 100 and $50\text{ }\mu\text{g}\cdot\text{mL}^{-1}$) diluted in Tween 80 at 2%. Groups V and VI corresponded to the negative control (with distilled water and Tween 80 at 2%) and the positive control (Amitraz at 12.5%), respectively. The experiment was conducted in triplicate.

To calculate mortality, the following formula was used: $\text{Mortality (\%)} = \text{Dead larvae} \times 100 / \text{Total larvae}$. The mean mortality was defined as the mean mortality (%) = repetition mortality 1 + repetition mortality 2 + repetition mortality 3.

The results were analyzed with Tukey test at 1% probability ($p < 0.001$), using the Statistical Package Sisvar (2015). To determine the lethal concentrations (LC) capable of causing 50% (LC_{50}) and 90% (LC_{90}) of larvae mortality, the *Probit* regression analysis was used, within a 95% confidence interval using *Statgraphics centurion XVI*, version 16.2.04 (2015).

3. Results and Discussion

3.1 Chemical composition of *Piper macedoi* essential oil

It was possible to identify 44 compounds in the chemical analysis of the essential oil of *Piper macedoi* leaves, distributed mainly among monoterpenes (12.78%), sesquiterpenes (17.20%) and arylpropanoids (68.43%). The major substances found were arylpropanoids apiole (39.81%) and dillapiole (26.47%), followed by the sesquiterpene bicyclogermacrene (4.88%) and monoterpenes (*E*)- β -ocimene (4.55%) and β -pinene (3.21 %) (Table 1).

Table 1: Chemical characterization of the *Piper macedoi* essential oil from Atlantic Forest of the South of Bahia, Brazil.

Substances	RI	RILit	Relative Percentage %
Tricyclene	931	926	1.02
β -Pinene	977	979	3.21
Myrcene	988	990	0.15
α -Phellandrene	999	1002	0.03
Limonene	1028	1029	0.20
(<i>Z</i>)- β -Ocimene	1033	1037	2.02
(<i>E</i>)- β -Ocimene	1044	1050	4.55
Linalool	1100	1096	0.42
Allocimene	1137	1132	0.23
Safrole	1288	1297	0.16
2-Undecanone	1293	1294	0.13
<i>Neo</i> Verbenol <i>Iso</i> -Acetate	1328	1330	0.98
β -Elemene	1386	1390	0.68
(<i>E</i>)-Caryophyllene	1416	1419	1.38
α -Humulene	1456	1454	0.27
Germacrene D	1485	1481	0.52
Valencene	1497	1496	0.12
Bicyclogermacrene	1497	1500	4.88
α -Muurolene	1500	1500	0.24
<i>n</i> -Pentadecane	1505	1500	0.10
Cubebol	1514	1515	1.23
Nootkatone	1517	1518	0.95

Mirysticin	1520	1518	1.56
<i>trans</i> -Cadina-1,4-diene	1528	1534	0.10
Elemicin	1544	1557	0.43
(E)-Nerolidol	1555	1563	1.56
Germacrene D-4-ol	1564	1575	1.24
α -Cedrene epoxide	1567	1575	0.17
Spathulenol	1570	1578	0.26
Presilphiperfolan-8-ol	1573	1583	0.16
Thujopsan-2- β -ol	1575	1589	0.26
Guaiol	1578	1600	0.13
β -Oplophenone	1586	1607	0.17
Humulene Epoxide II	1590	1608	0,20
Dilapiolle	1603	1620	26.47
10- <i>epi</i> - γ -Eudesmol	1612	1623	0.34
Eremoligenol	1622	16,31	0.11
<i>Epi</i> - α -Cadinol	1639	1640	0.30
<i>Epi</i> - α -Muurolol	1642	1642	0.48
Agarospirol	1647	1648	0.19
α -Cadinol	1662	1654	0.77
<i>cis</i> -Calamenen-10-ol	1693	1661	0.15
Apiole	1703	1678	39.81
Shyobunol	1712	1689	0.35
Monoterpenes			12.78
Sesquiterpenes			17.20
Arylpropanoids			68.43
Total %			98.41

Fonte: Autores (2021).

The chemical study of *P. macedoi* collected in an Atlantic Forest area is a pioneer work, and there are no reports in the literature of biological assays performed neither with extracts nor with essential oils of this species. However, chemical analyses of the essential oil of *P. macedoi* leaves were performed using specimens collected in a bush savanna (Cerrado) area in the State of Minas Gerais (Oliveira et al., 2016). This study showed that monoterpenes were identified as the major compounds, both for the wild plant sample (46.2%) as for a cultivated sample (46.7%), being (*E*)- β -ocimene the substance present in the highest concentration (wild plant 28.5%; cultivated plant 24.9%). The class of arylpropanoids also appeared in great percentual content (wild plant 25.3%; cultivated plant 23.5%), being partisan the majority compound (wild plant 14.1%; cultivated plant 17.8%). Dilapiolle was identified in this sample at low concentration (wild plant 1.1%; cultivated plant 1.8%) and apiole was not registered in the essential oil of *P. macedoi* from Cerrado site. The sesquiterpene fraction (wild plant 20.9%; cultivated plant 22.6%) was also found in reasonable content, with germacrene D as the major compound for this group (wild plant 3.7% and cultivated plant 3.2%) (Oliveira et al., 2016). The differences between the major compounds found in *P. macedoi* essential oil from the Atlantic Forest probably occur due to environmental and climatic differences.

3.2 *Piper macedoi* Yunch essential oil acaricidal activity

The essential oil from leaves of *P. macedoi* was shown to be effective, presenting an acaricidal activity against the larvae of *R. sanguineus*. After 24 h of larvae exposure to different essential oil concentrations, a mortality of 80.67% was observed at a concentration of 500 $\mu\text{g.mL}^{-1}$, presenting a result similar to the one observed for amitraz acaricide, which produced 82.33% of mortality. Concentrations of 250, 100, and 50 $\mu\text{g.mL}^{-1}$ showed mortality rates of 55.00%; 52.67% and 58.00%, respectively (Table 2).

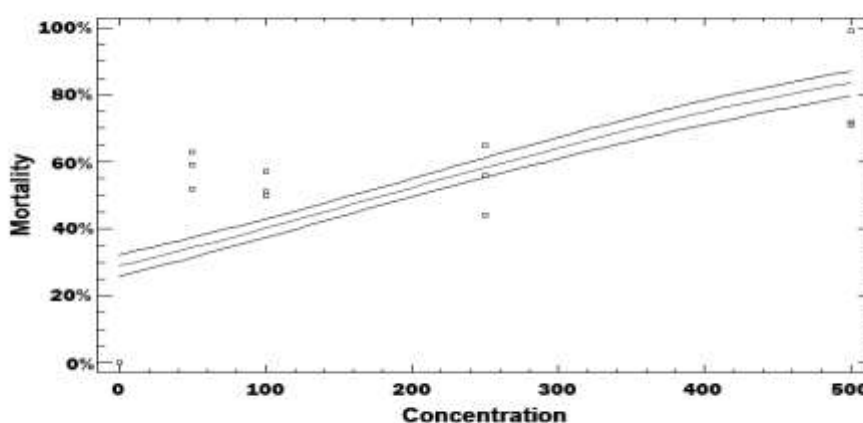
Table 2 - Percentage of mortality of *R. sanguineus* larvae exposed to different concentrations of *P. macedoi* essential oil.

Treatments ($\mu\text{g.mL}^{-1}$)	Mortality of larvae*
500	80.67 ^{ab}
250	55.00 ^c
100	52.67 ^c
50	58.00 ^{bc}
Amitraz 12.5%	82.33 ^a
Distilled water and Tween 80, 2%	0.00 ^d

*Averages followed by the same letters do not differ from each other by the Tukey test at 1 % probability ($p < 0.001$).
 Fonte: Autores (2021).

There was an increase in the efficacy of the mortality rate of *R. sanguineus* larvae proportional to the increase in the concentration of essential oil, indicating a dose-dependent response (Figure 1). The LC_{50} and LC_{90} for the essential oil of *P. macedoi* were calculated at 221.946 $\mu\text{g.mL}^{-1}$ and 557.084 $\mu\text{g.mL}^{-1}$, respectively (Figure 1). Thus, the data suggest a higher dose of essential oil to present a mortality rate of more than 90% and to present results higher than Amitraz.

Figure 1 - Variation in mortality of *R. sanguineus* larvae exposed to different concentrations of *P. macedoi* essential oil. Probit regression test with 95 % confidence interval.



Fonte: Autores (2021).

The drug of choice for the environmental treatment, where 95% of the tick population can be found, has long been based on pyrethroids because this class of acaricide has greater residual power (Labruna & Machado, 2006). However, recently, due to the increase in resistance to pyrethroids, these were replaced by formamidines (Amitraz), to which, in the same way, some tick populations are creating resistance (Farias et al., 2008; Santos et al., 2009). Thus, the search for new products,

especially of natural sources, is an alternative that should be considered. The results presented here, with similar activities between the essential oil of *P. macedoi* (500 $\mu\text{g}\cdot\text{mL}^{-1}$) and the drug Amitraz suggests the possibility of an alternative product to be used in environments and facilities where tick larvae are usually found, and even in the dog, after appropriate toxicological tests.

As observed in this study, the chemical composition of many essential oils, showing monoterpenes and sesquiterpenes, is responsible for repellency and biocide activities against the tick *R. (B.) microplus* (Facey et al., 2005; Gazim et al., 2011). These components, with arylpropanoids, especially represented by apiole, dillapiole, myristicin, safrole and sarisan, are found in species of the family Piperaceae, also show interesting biological properties (Santos et al., 2001, Salehi et al., 2019). Indeed, species of Piperaceae rich in arylpropanoids, such as *Piper aduncum* L., are also bioactive, demonstrating the activity against *Schistosoma* sp. As well as insecticide, larvicide and fungicide activities (Almeida et al., 2009). According to the literature, the major compounds of the essential oil of *P. aduncum* from Amazon region are the arylpropanoid dillapiole, responsible for most of the biological activities of the species (Almeida et al., 2009). Another study also conducted with *P. aduncum* showed excellent activity against *R. (B.) microplus*. The essential oil recovered from the hexane extract of this plant presented, in its chemical constitution, the arylpropanoid dillapiole as the main substance (94.84%) and an activity capable of causing the mortality of 100% of *R. microplus* larvae at low concentrations (0.1 $\text{mg}\cdot\text{mL}^{-1}$; 0.5 $\text{mg}\cdot\text{mL}^{-1}$; 1 $\text{mg}\cdot\text{mL}^{-1}$) (Silva et al., 2009).

Comparing the data presented in the literature and considering that in this study the essential oil of *P. macedoi* showed 66.28% of the chemical constitution of its essential oil represented by arylpropanoids (apiole - 39.81% and dillapiole - 26.47%), it is possible to associate the larvicidal activity with the presence of these compounds. A synergistic effect between them may be considered. In fact, the synergy between compounds from plant extracts and essential oils is one of the main advantages in the use of natural products in relation to synthetic drugs, as they can have more than one active ingredient acting synergistically, potentiating the biological activity in question, also hindering the formation of resistance of ticks, unlike synthetic products (Mgbojikwe & Okoye, 2004).

4. Conclusion

It was possible to conclude that the essential oil from fresh leaves of *P. macedoi* showed an activity against the larvae of *R. sanguineus*, with a better efficiency observed for concentrating 500 $\mu\text{g}\cdot\text{mL}^{-1}$, also indicating a dose-dependent response. Also, it is possible to suggest that the larvicidal activity against *R. sanguineus* presented here may be associated with apiole and dillapiole, not ruling out synergy between all compounds.

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References

- Adams, R.P. (2017). *Identification of essential oil components by gas chromatography/ mass spectrometry*. Allured Publishing Corporation: Carol Stream, Illinois, 809p.
- Almeida, R.R.P., Souto, R.N.P., Bastos, C.N., Silva, M.H.L., Maia, J.G.S. (2009). Chemical variation in *Piper aduncum* and biological properties of its dillapiole-rich essential oil. *Chemistry & Biodiversity*, 6, 1427-1434. <https://doi.org/10.1002/cbdv.200800212>.
- Araújo, R.P., Navarro M.B.M.A., Cardoso, T.A.O. (2015a). Spotted fever in Brazil: mortality study for epidemiological surveillance. *Caderno de Saúde Coletiva*, 23, 354-361. <https://doi.org/10.1590/1414-462X201500040094>.

- Araújo, A.C., Silveira, J.A.G., Azevedo, S.S., Nieri-Bastos, F.A., Ribeiro, M.F.B., Labruna, M.B., Horta, M.C. (2015b). Babesia canis vogeli infection in dogs and ticks in the semiarid region of Pernambuco, Brazil. *Pesquisa Veterinária Brasileira*, 35 (5), 456-461. <https://doi.org/10.1590/S0100-736X2015000500012>.
- Campos, R.N.S., Bacci, L., Araújo, A.P.A., Blank, A.F., Arrigoni-Blank, M.F., Santos, G.R.A., Roner, M.N.B. (2012). Óleos Essenciais de Plantas Medicinais e Aromáticas no Controle do Carrapato *Rhipicephalus Microplus*. *Archivos de Zootecnia*, 61, 67-78. <https://doi.org/10.21071/az.v61i237.2959>.
- Chagas, A.C.S. (2004). Controle de parasitas utilizando extratos vegetais. *Revista Brasileira de Parasitologia Veterinária*, 13, 156-160.
- Dantas-Torres, F., Figueredo, L.A. (2006). Canine babesiosis: a Brazilian perspective. *Veterinary Parasitology*, 141, 197-203. <https://doi.org/10.1016/j.vetpar.2006.07.030>.
- Facey, P.C., Porter, R.B.R., Reese, P.B., Williams, L.A.D. (2005). Biological activity and chemical composition of the essential oil from Jamaican *Hyptis verticillata* Jacq. *Journal of Agricultural and Food Chemistry*, 53, 4774-4777. <https://doi.org/10.1021/jf050008y>.
- Farias, N.A., Ruas, J.L., Santos, T.R.B. (2008). Análise da eficácia de acaricidas sobre o carrapato *Boophilus microplus*, durante a última década, na região sul do Rio Grande do Sul. *Ciência Rural*, 38 (6), 1700-1704. <http://dx.doi.org/10.1590/S0103-84782008000600032>.
- Gazim, Z.C., Demarchi, I.G., Lonardon, M.V.C., Amorim, A.C.L., Hovell, A.M.C., Rezende, C.M., Ferreira, G.A., Lima, E.L., Cosmo, F.A., Cortez, D.A.G. (2011). Acaricidal activity of the essential oil from *Tetradenia riparia* (Lamiaceae) on the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Acari; Ixodidae). *Experimental Parasitology*, 129, 175-178. <https://doi.org/10.1016/j.exppara.2011.06.011>
- Labruna, M.B., Paula, C.D., Lima, T.F., Sana, D.A. (2002). Ticks (Acari: Ixodidae) on wild animals from the Porto Primavera Hydroelectric power station area, Brazil. *Memoria Institucional Oswaldo Cruz*, 97, 1133-1136. <https://doi.org/10.1590/S0074-02762002000800012>
- Labruna, M.B., Machado, R.Z. (2006). Agentes transmitidos por carrapatos na região neotropical. In: Barros-Batesti, D.M., Arzua, M., Bechara, G.H. *Carrapatos de importância médico-veterinária da região neotropical, um guia ilustrado para identificação de espécies*. São Paulo: Instituto Butantan, 115-138.
- Lambert, M.M., Chaves, D.S.A., Avelar, B.R., Campos, D.R., Borges, D.A.B., Moreira, L.O., Pereira, G.A., Cid, Y.P., Scott, F.B., Coumendouros, K. (2021). In vitro evaluation of the acaricidal activity of *Syzygium aromaticum* (L.) essential oil and eugenol against non-fed larvae of *Rhipicephalus sanguineus* Brazilian *Journal of Veterinary Medicine*, 43, e002620. <https://doi.org/10.29374/2527-2179.bjvm002620>
- Louly, C.C.B., Fonseca, I.N., Oliveira, V.F., Borges, L.M.F. (2006). Ocorrência de *Rhipicephalus sanguineus* em Trabalhadores de Clínicas Veterinárias e Canis, no município de Goiânia, GO. *Ciência Animal Brasileira*, 7, 103-106.
- Martins, T.F., Spolidorio, M.G., Batista, T.C.A., Oliveira, I.A.S., Natalino, H., Yoshinari, N.H., Labruna, M.B. (2009). Ocorrência de carrapatos (Acari: Ixodidae) no município de Goiatins, Tocantins. *Revista Brasileira de Parasitologia Veterinária*, 18, 50-52. <https://doi.org/10.4322/rbvp.01802011>.
- Mantovani E.; Costa, I.P.; Gauditano, G.; Bonoldi, V.L.N.; Higuchi, M.L.; Yoshinari, N.H. (2007). Description of Lyme disease-like syndrome in Brazil. Is it a new tick borne disease or Lyme disease variation? *Brazilian Journal of Medical and Biological Research*, 40, 443-456. <https://doi.org/10.1590/S0100-879X2006005000082>.
- Massard, C.L., Fonseca, A.H. (2004). Carrapatos e doenças transmitidas comuns ao homem e aos animais. *Hora Veterinária*, 135, 15-23.
- Mentz, M. B., Trombka, M., Silva, G. L., & Silva, C. E. (2016). *Rhipicephalus sanguineus* (ACARI: IXODIDAE) biting a human being in Porto Alegre city, Rio Grande do Sul, Brazil. *Revista do Instituto de Medicina Tropical de Sao Paulo*, 58, 35. <https://doi.org/10.1590/S1678-9946201658035>
- Mgbojikwe, L.O., Okoye, S.C. (2001). Acaricidal Efficacy of the Aqueous Stem Bark Extract of *Adenium obesum* on the Various Life Stages of Cattle Ticks. *Nigerian Journal of Experimental and Applied Biology*, 2, 39-43. <https://doi.org/10.4102/ojvr.v86i1.1665>
- Moraes-Filho, J. Pinter A, Pacheco RC, Gutmann TB, Barbosa SO, Gonzáles MA, Muraro M.A, Cecílio SR, Labruna MB. (2009). New epidemiological data on Brazilian spotted fever in an endemic area of the state of São Paulo, Brazil. *Vector Borne Zoonotic Diseases*, 9, 73-78. <https://doi.org/10.1089/vbz.2007.0227>
- Nwanade, C.F., Wang, M., Wang, T., Yu, Z., Liu, J. (2020). Botanical acaricides and repellents in tick control: current status and future directions. *Experimental and Applied Acarology*, 81, 1-35. <https://doi.org/10.1007/s10493-020-00489-z>.
- Oliveira GL, Moreira DL, Mendes ADR, Guimarães EF, Figueiredo LS, Kaplan, M.A.C.; Martins, E.R. (2013). Growth study and essential oil analysis of *Piper aduncum* from two sites of Cerrado biome of Minas Gerais State, Brazil. *Revista Brasileira de Farmacognosia*, 23, 743-753. <https://doi.org/10.1590/S0102-695X2013000500005>.
- Oliveira, G.L., Matos, C.C., Santos, R.R., Maia, J.T.S., Martins, E.R., Figueiredo, L.S., Moreira, D.L., Kaplan, M.A.C. (2016). Rooting of cuttings and analysis of essential oils from wild and cultivated *Piper macedoi* Yunck. *Revista Brasileira de Plantas Medicinais*, 18, 782-790.
- Olivo, C.J., Carvalho, N.C., Silva, J.H., Vogel, F.F. Massariol P, Meinerz G, Agnolin C, Morel AF, Viau LV. (2008). Óleo de citronela no controle do carrapato de bovinos. *Ciência Rural*, 38, 406-410. <http://dx.doi.org/10.1590/S0103-84782008000200018>.
- Salehi, B., Zakaria, Z.A., Gyawali, R., Ibrahim, S.A., Rajkovic, J., Shinwari, Z.K., Khan, T., Sharifi-Rad, J., Ozleyen, A., Turkdonmez, E., Valussi, M., Tumer, T.B., Fidalgo, L.M., Martorell, M., Setzer, W.N. (2019). Piper Species: A Comprehensive Review on Their Phytochemistry, Biological Activities and Applications. *Molecules*, 24, 1364. <https://doi.org/10.3390/molecules24071364>
- Santos, P.R.D., Moreira, D.L., Guimarães, E.F. Kaplan, M.A.C. (2001). Essential oil analysis of 10 Piperaceae species from the Brazilian Atlantic forest. *Phytochemistry*, 58, 547-551. [https://doi.org/10.1016/S0031-9422\(01\)00290-4](https://doi.org/10.1016/S0031-9422(01)00290-4).

Santos, T.R.B., Pappen, F.G., Faria, N.A.R., Vaz Junior, I.S. (2009). Análise in vitro da eficácia do amitraz sobre populações de *Rhipicephalus (Boophilus) microplus* (Canestrini, 1887) da região sul do Rio Grande do Sul. *Revista Brasileira de Parasitologia Veterinária*, 18, 54-57. <https://doi.org/10.4322/rbpv.018e1010>.

Silva, W.C., Martins, J.R.S., Souza, H.E.M., Heinzen, H., Cesio, M.V., Mato, M., Albrecht, F., Azevedo, J.L., Barros, N.M. (2009). Toxicity of *Piper aduncum* L. (Piperales: Piperaceae) from the Amazon forest for the cattle tick *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae). *Veterinary Parasitology*, 164, 267-274.

Vélez, J.C.Q., Hidalgo, M., González, J.D.R. (2012). *Rickettsiosis, una enfermedad letal emergente y re-emergente en Colombia*. Universitas Scientiarum. 17, 82-99. <http://dx.doi.org/10.11144/javeriana.SC17-1.rade>

Veríssimo, C.J., Katiki, L.M. (2015). *Alternativas de controle do carrapato-do-boi na pecuária leiteira. Resistência e Controle do Carrapato-do-boi*. Nova Odessa: Instituto de Zootecnia, 113 p.

Yoshinari, N.H., Mantovani, E., Bonoldi, V.L.N., Marangoni, R.G., Gauditano, G. (2010). Doença de lyme-símile brasileira ou síndrome baggio-yoshinari: zoonose exótica e emergente transmitida por carrapatos. *Revista da Associação Médica Brasileira*, 56, 363-9. <https://doi.org/10.1590/S0104-42302010000300025>.