Acaricide effect of native and exotic plants from the Brazilian savanna on Rhipicephalus microplus-resistant ticks with notes on the acaricide resistance of tick populations collected in southeast Brazil

Maykelin Fuentes Zaldivar
Universidade Federal de Minas Gerais
Eduardo Bastianetto
Universidade Federal de Minas Gerais
Adalberto Alves Pereira Filho
Universidade Federal de Minas Gerais
Daniel Sobreira Rodrigues
Santa Rita Experimental Field, Empresa Mineira de Pesquisa Agropecuária de Minas Gerais, Minas Gerais
Valdo Soares Martins Júnior
Universidade Federal de Minas Gerais
Viviane Oliveira Vasconcelos
Universidade Estadual de Montes Claros
Eduardo Robson Duarte
Universidade Federal de Minas Gerais
Ricardo Nascimento Araujo
rnaraujo@icb.ufmg.br

Universidade Federal de Minas Gerais

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Abstract

Rhipicephalus microplus is among the most important ectoparasites for livestock. The use of chemical acaricides has raised some concerns due to the selection of tick populations that are resistant to acaricides and environmental contamination. Therefore, plant extracts have been used as alternatives for the treatment of animals infested with ticks. In this study, *R. microplus* populations from seven different dairy farms were collected and assessed for their resistance to acaricides. Larvae of the most resistant population was used in assays to evaluate the acaricide effect of extracts from leaves or the latex of eight plant species commonly found in Brazilian dairy farms. The most active extracts were also tested against fully engorged females. Among seven tick populations, five and three showed resistance level ≥ III for cypermethrin or trichlorfon, respectively. The most resistant population was used in mortality assays with the plants *Piptadenia viridiflora*, *Annona crassiflora*, *Caryocar brasiliense*, *Ximenia americana*, *Schinopsis brasilienses*, *Mangifera indica*, *Tithonia diversifolia*, and *Calotropis procera*. *C. procera* was the most effective against larvae with mortalities of 62.9 and 44.9% at 100 mg/mL with the latex and aqueous extract, respectively. The ethanolic extracts of *C. brasiliense*, *S. brasilienses*, and *X. americana* also had acaricide effects higher or similar to cypermethrin. When fully engorged females were tested, the aqueous extract of *C. procera* showed a 96.8% efficiency. Four of the plants tested showed promising results, and further studies should be carried out for the development of tools to control *R. microplus* in cattle.

Introduction

Rhipicephalus (*Boophilus*) *microplus* is the main ectoparasite of cattle worldwide, causing significant economic impact on livestock (Pereira et al. 2022). It causes losses related to animal mortality, reduction in cattle productivity levels, reproductive disorders, and transmits different pathogens to their hosts. In addition, the hematophagy in animals causes skin lesions, which can be a “gateway” for secondary infections and myiasis (Jonsson 2006; de la Fuente et al. 2008; Rodrigues and Leite 2013; Grisi et al. 2014).

Tick control programs are based on the use of chemical products such as pyrethroids and organophosphates. Despite their notorious benefits, the intense and often inappropriate use may exert selective pressure on tick populations and contribute to the emergence of resistant strains (George et al. 2004; Klafke et al. 2017; Dolenga et al. 2022). Additionally, residues of those chemical acaricides can contaminate animal products such as milk and meat, the environment, and workers dealing with animals (Laing et al. 2018; Welsh et al. 2019).

Organic control strategies based on the use of phytotherapy has gained more acceptability over time, as it is safer since they tend to degrade quickly in the environment and tend not to accumulate as residues in beef and milk (Adenubi et al. 2016; Quadros et al. 2020). For *R. microplus*, several studies have shown the acaricidal properties of a wide variety of plant leaf extracts (Shyma et al. 2014; Vasconcelos et al. 2018; Wadhwani et al. 2021). In addition, botanical compounds present in plants, such as carvacrol and thymol, are effective against tick strains that are resistant to synthetic acaricides (Costa-Júnior et al. 2016).

The savanna biome in southeast Brazil has a rich abundance of tree and shrub plants such as *Piptadenia viridiflora* Kunth (Fabaceae), *Annona crassoflora* Mart (Annonaceae), *Caryocar brasiliense* Camb. (Caryocaraceae), *Ximenia americana* L. (Olacaceae), *Schinopsis brasilienses* Engl. (Anacardiaceae), *Mangifera indica* L. (Anacardiaceae), *Tithonia diversifolia* (Hemsl., Asteraceae), and *Calotropis procera* (Aiton, Apocynaceae) (Silva Junior, 2012). These plants have diverse medical proprieties, and it is noteworthy that some of them, such as *P. viridiflora*, *X. americana*, and *M. indica*, are species that are selected by grazing sheep (Elango and Rahuman 2011; Morais-Costa et al. 2015; Wadhwani et al. 2021). Extracts from some of those plants were described to have an anthelmintic action on

Haemonchus contortus (Morais-Costa et al. 2015), molluscicidal on *Biomphalaria glabrata* (Cruz et al. 2022), insecticide on *Ctenocephalides felis* and *Ctenocephalides canis* (Maina et al. 2018), and to also have antibacterial potential (Maikai et al. 2009).

In ticks, a previous study showed that extracts of *P. viridiflora*, *X. americana*, and *S. brasilienses* were able to affect reproductive parameters of engorged females of the tick, *Dermacentor nitens* (Vasconcelos et al. 2018). Extracts of the exotic plant, *C. procera*, were also effective against larvae and engorged females of *R. microplus* (Shyma et al. 2014; Khan et al. 2019); however, information about the effects of plant extracts in resistant *R. microplus* are still scarce in the literature. Therefore, the objective of this study was to identify the cypermethirn and triclorfone resistance levels of different *R. microplus* populations and to assess the effects of leaf extracts and the latex of native and exotic plants of the savanna biome on larvae of the most resistant tick population. The plant products with higher activities had its acaricide effect also evaluated on fully engorged females of the same resistant population.

Methodology

Ticks

Rhipicephalus microplus colonies of sensitive (Porto Alegre) and resistant (Jaguar) reference strains were maintained in cattle at the facilities of the Laboratory of Parasitology of the ICA/UFMG at Montes Claros, MG, Brazil. Field strains were collected between June 2018 and March 2019 form different dairy farms located at the state of MG, Brazil, as follows: Funilândia (S19°22'4",W44°3'21"), Moema (S19°51'5", W45°24'45"), Bocaiuva (S17°6'41.06",W43°49'13.53"), Santa Rita (S19°27'31.6", W44°09'44.6"), Boa Vitória (S18°50'25.504", W50°7'16.47"), Igarapé (S20°4'16.28", W44°17'59.82"), and Nova Esperança (S16°51'17.05", W43°50'24.19"). Ticks were collected from naturally infested Gir x Holstein crossbred cattle that have not received acaricide for the last 60 days. The collected ticks were transferred to the laboratory where females larger than 4 mm were selected, washed with distilled water, dried on paper towels, and kept on petri dishes for oviposition or use in the experiments.

Plant collection

Plants were collected between April and June 2017 from dairy farms in the rural area of Montes Claros, Brazil (S16°41'10.05°, W43°50'33.56"). The climate of the region is humid and tropical with dry summers, according to the Köppen classification (Alvares et al. 2013). The collection area consists of savanna vegetation with fields in various stages of succession due to the grazing of domestic animals. Collected plants were registered in SISGEN with numbers A95A7D4, AFA7072, A75FA55, and AD861E4. Representative parts and the respective vouchers were identified (Lorenzi 2002) and deposited in the Herbarium of the Universidade Estadual de Montes Claros. Plants used in experiments are described in Table 1.

Table 1
Plant species collected at the municipally of Montes Claros, Brazil and the preparations used for the experiments.

Plants	Vernacular name	Family	Voucher	Preparations tested[a]
Native species				
Piptadenia viridiflora Kunth	Surucucu	*Fabacea*	2283	EE-EA
Annona crassiflora Mart.	Panã	*Annonaceae*	3475	EE
Caryocar brasiliense Camb.	Pequi	*Caryocaraceae*	338	EE
Ximenia americana L.	Ameixa do Cerrado	*Olacaceae*	211	EE-EA
Schinopsis brasilienses Engl.	Brauna	*Anacardiaceae*	377	EE
Exotic species				
Mangifera indica L.	Manga	*Anacardiaceae*	-	EE
Tithonia diversifolia (Hemsl.)	Girassol Mexicano	*Asteraceae*	-	EA
Calotropis procera (Aiton)	Algodão de Seda	*Apocynacea*	-	Latex - EA

[a]Aquous extract (EA); Ethanolic extracts (EE).

Preparation of plant extracts and latex

The leaves were separated from the rest of the plant and were dehydrated in an oven with forced air circulation (TE 394/4, Tecnal Equipamentos Científicos) at 38ºC for 72 hours. They were then crushed using an industrial blender in order to obtain a dry powder that was stored in paper bags. The aqueous extract (EA) was produced by immersing the dry powder in distilled water in a water bath at 40°C for 60 minutes. The ethanolic extracts (EEs) were prepared by immersion in absolute ethanol PA 99.5 ºGL, incubation at room temperature for 10 days, and filtration in glass funnels with gauze. EA and EE were dehydrated in a forced-circulation oven at 40 ± 5ºC for up to 72 hours, and then stored in dark bottles at 4ºC. The latex of *C. procera* was obtained by removing the leaves and collecting the natural exudation at the base of the stem of the plant. The material was subsequently dehydrated in an oven at 40ºC for three days and stored in dark bottles at 4ºC. Aliquots of extracts and latex were taken for the determination of dry matter at 105°C (Association of official analytical chemists 1990).

Larval packet test (LPT)

The LPT (FAO, 2004) was used to assess the acaricide resistance from each population and to describe the acaricide activity of the plant products. Resistance to α-Cypermethrin (BASF) and trichlorfon (Neguvon®, Bayer) was assessed using the discriminating dose of 0.002 and 0.99 mg/mL, respectively, diluted in tricloroetilene P.A. (Synth) and commercial olive oil (2:1; v:v). For LC50 and LC90 calculations, 16 dilutions of each acaricide ranging from 0.009 to 12% were used. The diluents without acaricides were used as a negative control. Plant products were evaluated at 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78 mg/mL with distilled water as a negative control. Five repetitions of each treatment were carried out. Filter papers (Whatmann nº1) with 8.5 × 7.5 cm were treated with 0.67 mL of each acaricide concentration, dried for 24 hours at room temperature, and then stored at 4°C wrapped in aluminum papers until use.

For the assays, approximately 100 larvae at 14–21 days were added to the papers, which were folded in half, closed with clips on each side, arranged on plastic trays, and kept at 27°C for 24 hours. After incubation, the number of dead and alive larvae were counted using a vacuum pump (Primar, 141/2VC). Larvae unable to move were considered dead.

Adult immersion test (AIT)

Assays were carried out according to Drummond et al. (1973). Briefly, five fully engorged females were immersed for 5 minutes in flasks containing 5 mL of each plant solution at the concentrations of 25, 50, and 100 mg/mL. Females were then dried with paper towels, transferred to petri dishes (90 × 10 mm diameter-height), and then kept in incubators at 28 ± 2°C and 70 ± 10% relative humidity. Assays were carried out in 5 repetitions containing 10 ticks each. Distilled water was used as a negative control.

Females were kept in incubators for 15 days, after which the mass of eggs were weighed, transferred to 3 mL syringes with an open end that was plugged with cotton, and then stored in incubators at the conditions above. Larval hatching (H) was measured after four weeks by transferring the whole content of the syringe into a flask containing water and commercial detergent (1:1, v:v), which was stirred. Three aliquots were transferred to glass slides so that the larvae, egg shells, and non-viable eggs could be counted under a microscope at 100X.

The egg-laying-capacity (ELC) was determined by dividing the weight of the egg mass by the initial weight of the female and multiplying the result by 100 (adapted from Bennett 1974). Treatment efficiency was determined by adapting the formula proposed by Drummond et al. (1973) as follows: reproductive efficiency (RE) = (egg mass weight × % hatching × 20,000) / female initial weight; product efficacy (E) = (Control RE - Product RE / Control RE) × 100.

Analysis of data

A field population was considered resistant when the mortality rate in the DD was less than 95%. Four resistance levels were established after quartile analysis according to Klafke et al. (2017) as follows: Sensible: mortality > 95%; resistance level I: 95% > mortality ≥ 82%; resistance level II: 82% > mortality ≥ 57%; resistance level III: 57% > mortality ≥ 25%; and resistance level IV: mortality < 25%. Additionally, a completely randomized design was used to compare the efficiency of extracts and latex with controls. Data were transformed into relative numbers and subjected to analysis of variance (ANOVA) and means compared by Duncan's test ($p \leq 0.05$) using the SAEG statistical package. 9.1.

Results

Acaricide resistance of tick populations

Porto Alegre and Jaguar reference strains had mortalities always above 95% or below 25% after treatment with cypermethrin and trichlorfon, respectively, confirming their acaricide susceptibility and resistance (Tables 2 and 3). Among the seven populations collected from farms in MG, all showed at least resistance level I to either cypermethrin (Table 2) or trichlorfon (Table 3). Ticks were more resistant to cypermethrin, as four populations achieved the highest resistance level (IV) and one achieved resistance level III. Concerning trichlorfon, three of the populations had resistance level III. Boa Vitoria was the population with the highest acaricide resistance, which was level IV for cypermethrin and level III for trichlorfon, and was selected for further experiments.

Acaricide effect of the plant extracts against larvae

Ethanolic and AE from five native plants from the savanna of MG were tested against the Boa Vitoria population. All of them had an acaricide effect that was significantly higher ($p < 0.05$) than cypermethrin in at least one concentration (Table 4). Only the EEs from *C. brasiliense*, *S. brasiliensis,* and *X. americana* presented acaricidal activity above 25%, while the other plant extracts demonstrated activities below 11% larval mortality, which did not demonstrate a relevant acaricide effect (Table 4).

The EE of *C. brasiliense* showed the highest effect, starting with 25% for the lowest concentration (0.78 mg/mL) up to 32.93% at 100 mg/mL. EE of *S. brasiliensis* and *X. americana* showed acaricide effects over 18% only at concentrations of 50 and 100 mg/mL (Table 4).

As for the products from the exotic plants, *C. procera* presented a mortality greater than 40%, but this was only achieved at the concentrations of 100 mg/mL for the AE and above 12.5 mg/mL for the latex (Table 5). Both AE and latex extracts of *C. procera* showed a dose-dependent effect. The *C. procera* latex was the most effective for larvae mortality, with 51.21 and 62.92% at the concentrations of 50 and 100 mg/mL, respectively. The other extracts demonstrated mortality rates of less than 10%, which demonstrates a low efficacy against resistant larvae of *R. microplus* (Table 5).

Acaricide effect of the plant extracts against fully engorged females

The extracts from the plants *C. procera*, *S. brasiliensis,* and *X. americana*, which were among the ones with the best efficiency against larvae, were then tested on fully engorged females. In general, extracts from all three plants were able to significantly reduce both egg production and H at 100 mg/mL in comparison to controls (distilled water) ($p < 0.05$) (Table 6). *C. procera* AE showed the highest efficiency, which was greater than 95% at the concentration of 25 mg/mL and almost 90% at 50 mg/mL, with both concentrations showing acaricide efficiency significantly higher than cypermethrin ($p < 0.05$) (Table 6). *S. brasiliensis* and *X. americana* were able to significantly affect the ELC and H in relation to controls, especially at the highest concentrations ($p < 0.05$); however, the acaricide efficiency of these plants was always low (below 35%, except for *X. americana* at 100 mg/mL) and lower than cypermethrin for all concentrations tested (Table 6).

Discussion

Chemical acaricides to control *R. microplus* have become limited in cattle farming over time, and due to the high selective pressure, resistant strains have been seen in several dairy farms (Higa et al. 2015; Klafke et al. 2017). Therefore, a more sustainable, less harmful, and more effective alternative approach is needed to control *R. microplus*. One of the most promising strategies is the identification and characterization of plants from which to obtain extracts with acaricidal properties, especially when dealing with resistant tick strains (Borges et al. 2011; Ghosh et al. 2015). Along with the variety of actions of herbal medicines on parasites when compared to synthetic products, herbal medicines are less toxic to mammals and the environment and are less likely to induce the selection of resistant strains (Wanzala 2017). This information suggests the need for the discovery of new botanical specimens that are potentially useful for the control of *R. microplus*.

In the present study, we showed that tick populations resistant to cypermethrin and trichlorfon, two common active ingredients used in acaricides against *R. microplus*, are a common finding in dairy farms of southeast Brazil. In addition, the effect of extracts or the latex of eight plants commonly found in this region were tested against a cypermethrin-resistant population, and four of them showed acaricide efficiency with a potential to be used as alternative controls.

As expected, the sensitivity and resistance level of the Porto Alegre and Jaguar reference strains were classified as sensitive and resistance level IV, respectively. The other seven tick populations from MG dairy farms were classified as resistance levels I to IV (Klafke et al. 2017), confirming previous work that acaricide-resistant populations are a common finding in MG (Domingues et al. 2012; Faza et al. 2013) and other states of Brazil (Higa et al. 2015, 2016). Resistance levels from III on indicate that there is a low effect of the acaricide, and that it should not be used against the tick population (Higa et al. 2015). Therefore, cypermethrin and trichlorfon are no longer indicated for use for tick control on 71.4 and 42.9%, respectively, of the farms in the present work.

The Boa Vitoria field population was selected for further experiments using plant products, as it was the one with the highest level of acaricide resistance. It is of note that Boa Vitoria ticks were collected from Marchigiana (*Bos taurus taurus*) cattle on a farm that used endectocide drug treatments for many years without technical supervision, which is a drug that is based on doramectin salt and other formulations containing pyrethroids and organophosphates. At that farm, doramectin-resistant *H. contortus* were also identified, and along with other ectoparasites, incriminated as causing the death of adult animals (non-published data). The parasitic control used at the farm suggests the presence of multi-resistant ticks.

Extracts derived from the species *C. brasiliense*, *S. brasiliensis*, *X. americana*, and *C. procera* and the latex of *C. procera* showed the highest efficiency against *R. microplus* larvae, with mortality rates varying between 25 and 62% at 100 mg/mL. The native species with the greatest effectiveness was *C. brasiliense*, while *C. procera* was the best among the exotic ones tested. They showed greater acaricidal potential against the strains that were resistant to pyrethroids and organophosphates from the Boa Vitoria farm. The extracts of *C. procera*, *S. brasliensis*, and *X. americana* plants reduced the ELC and H rate of *R. microplus* females. It is of note that the acaricidal efficiency of *C. procera* EA at a concentration of 25 mg/mL showed an efficacy of 96.8%, which was significantly higher than the cypermethrin efficiency.

Calotropis procera is an exotic plant with several known medicinal proprieties and activities against arthropods (Wadhwani et al. 2021). Several studies were carried out with ticks. In a previous study, the EE of *C. procera* induced a 63.2% mortality of *R. microplus* larvae at 100 mg/mL (Shyma et al. 2014), which is a percentage similar to the one found here. On the other hand, Khan et al. (2019) used 21.15 mg/mL and described a lethal effect of 90%, which is a considerably higher acaricidal activity in relation to the present work. Notably, these authors used whole parts of the *C. procera* plant, which may have likely increased the acaricidal activity against larvae. In addition, ticks used in the previous studies were not characterized as resistant to acaricides.

It is interesting to note that the lower concentration of the *C. procera* AE (25 mg/mL) was the one with highest efficiency (Table 6). This finding is in line with previous studies that observed that the highest concentration is not necessarily the one that induced the highest activity against fully engorged females. Lazaro et al. (2012) carried out AITs to evaluate the effect of *C. procera* EA on the reproductive parameters of *R. microplus* and observed an acaricidal efficiency of 98.3, 97, and 96% at the concentrations of 5, 25, and 100%, respectively. The effects of *C. procera* against *R. microplus* may reflect the presence of different active compounds in different combinations. The exact components present in the extracts and the latex used in the present work were not assessed, but previous work has shown the presence of cardenolides, flavonoids, sterols, oxypregnanes, triterpenoids, glycosides, among others, with a prevalence of flavonoids and glycosids in the leaves and steroids and cardenolides in the latex (Wadhwani et al. 2021). Indeed, Bhaskar et al. (2021) found that the AE of *C. procera* contained 3-Isopropoxy-1,1,1,7,7,7-hex as a major compound, and the AE showed a tick-killing effect against adult *R. microplus* of up to 93.33% at concentrations up to 360 mg/mL.

Extracts from the plants *S. brasiliensis*, *X. americana*, and *C. brasiliense* also had interesting acaricidal activities on *R. microplus* larvae at 100 mg/mL, which has not been described in the literature so far. In another study, extracts of *X. americana* and *S. brasiliensis* significantly reduced the ELC and H of the tick *D. nitens*, showing an acaricide efficiency above 75% at 100 mg/mL (Vasconcelos et al. 2018). In addition to the acaricidal activity, the extracts have also been demonstrated to have antimicrobial, insecticide, and anthelminthic activity (Maikai et al. 2009; Santos et al. 2014; Morais-Costa et al. 2015; Morais et al. 2020; Linhares et al. 2022). The composition of the EE of *S. brasiliensis* and *X. americana* used in the present work was characterized in a previous work (Vasconcelos et al. 2018). The extracts contained a predominance of polyphenols with a major presence of tannins in *S. brasiliensis* and tannins and flavonoids in *X. americana*. Leaf EE of *C. brasiliense* was also previously characterized, with flavonoids as the major compound (Morais et al. 2020), but the plants were collected in a different site and the real composition of the EE used here remains to be characterized. Nonetheless, tannins and flavonoids have been reported to have acaricidal activities (Fernández-Salas et al. 2011; Madzimure et al. 2011) and are believed to be the main component inducing the mortality of larvae.

Although *M. indica* had low acaricidal activity in the present work, previous studies have shown that its leaf AE can induce up to a 54% mortality on *R. microplus* larvae and up to a 100% mortality on other tick species and mosquito larvae (Rajakumar et al. 2015). A similar effect occurred for *P. viridiflora*, whose EE was previously shown to contain tannins and showed an efficiency of up to 98.7% against *D. nitens* females (Vasconcelos et al. 2018) and an anthelminthic action against *H. contortus* larvae (Morais-Costa et al. 2015); however, it is important to note that variations of the activity of similar plant species is a common finding. Such differences may be due to the location, collection method, extraction, and processing of the plants, which may influence the concentration of a given bioactive compound (Gobbo-Neto and Lopes 2007).

The differences in the plant acaricide efficiency between the present work and previous literature may also be due to the resistant tick strains used in the experiments. Acaricide-resistant ticks may have genetic and physiological differences in cuticle production and detoxification enzymes, among others (De Rouck et al. 2023), that could influence the cuticle penetration and detoxification of the products. A previous work has reported that acaricide-resistant *R. microplus* have a lower reproductive performance (Davey et al. 2006) and a higher susceptibility to carvacrol (Costa-Júnior et al. 2016), which is a monoterpene commonly found in the essential oils of plants with acaricide effects against ticks (Quadros et al. 2020).

The findings of the present work, in addition to those available in previous literature, encourages future investigations of the effect of plants from the Brazilian savanna against ticks, and also suggest that further evaluation on the effect of *C. procera* is needed. The identification of the bioactive molecules responsible for the tick-killing effects of *C. procera* could be useful for the generation of a formulation that optimizes the effects of these molecules. Furthermore, it is essential to determine whether the properties of *C. procera* are restricted to ticks and if they are potentially harmful to other animals, including humans.

Conclusion

Among the seven *R. microplus* populations from dairy farms of MG, Brazil, five and three showed resistance level ≥ III to cypermethrin or trichlorfon, respectively; however, among the eight plants tested on larvae and fully engorged *R. microplus* females that were resistant to cypermethrin and trichlorfon, four extracts presented acaricidal activity on larvae (*S. brasiliensis*, *X. americana*, *C. brasiliense*, and *C. procera*). The AE of *C. procera* and the EE of *X. americana* are promising alternatives for the treatment of animals parasitized with resistant *R. microplus* populations.

Declarations

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Competing interests The authors declare no competing interests.

Author Contributions

RNA and ERD designed the study; EB and DSR collected the tick field populations; ERD, VVO and DSR maintained the reference tick strains; MFZ, APAF, EB, DSR performed the tick mortality assays with active ingredients; VVO, VSMJ and ERD collected the plants and prepared the extracts and the latex; MFZ, APAF performed the tick mortality assays with plant extracts and the latex; RNA, MFZ and ERD wrote the manuscript. All authors analyzed the data, read and approved the final manuscript.

Data Availability

Data generated during the current study are available from the corresponding author on reasonable request.

References

1. Adenubi OT, Fasina FO, McGaw LJ, et al (2016) Plant extracts to control ticks of veterinary and medical importance: A review. South Afr J Bot 105:178–193. https://doi.org/10.1016/j.sajb.2016.03.010
2. Alvares CA, Stape JL, Sentelhas PC, et al (2013) Köppen's climate classification map for Brazil. Meteorol Z 22:711–728
3. Association of official analytical chemists (1990) Official Methods of Analysis, 15th edn. Kenneth Helrich, Arlington
4. Bennett GF (1974) Oviposition of Boophilus microplus (Canestrini) (Acarida : Ixodidae). I. Influence of tick size on egg production. Acarologia 16:52–61
5. Bhaskar S, Sharma V, Rahal A (2021) Acaricidal Bioefficacy of Calotropisprocera (Asclepiadaceae) of Middle Gangetic Region against Cattle Tick, *Rhipicephalus microplus* and Their Gc-Ms Analysis. 25:1795 - 1809
6. Borges LMF, Sousa LAD, Barbosa CS (2011) Perspectives for the use of plant extracts to control the cattle tick *Rhipicephalus* (*Boophilus*) *microplus*. Braz J Vet Parasitol. 20:89–96. https://doi.org/10.1590/s1984-29612011000200001

7. Costa-Júnior LM, Miller RJ, Alves PB, et al (2016) Acaricidal efficacies of *Lippia gracilis* essential oil and its phytochemicals against organophosphate-resistant and susceptible strains of *Rhipicephalus* (*Boophilus*) *microplus*. Vet Parasitol 228:60–64. https://doi.org/10.1016/j.vetpar.2016.05.028
8. Cruz JP, Ribeiro F, Vasconcelos VDO (2022) Molluscicidal activity of extracts of plants from the Cerrado against *Biomphalaria glabrata* (Say, 1818). Res Soc Dev 11:e20611830656. https://doi.org/10.33448/rsd-v11i8.30656
9. Davey RB, George JE, Miller RJ (2006) Comparison of the reproductive biology between acaricide-resistant and acaricide-susceptible *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae). Vet Parasitol 139:211–220. https://doi.org/10.1016/j.vetpar.2006.02.027
10. de la Fuente J, Estrada-Pena A, Venzal JM, et al (2008) Overview: Ticks as vectors of pathogens that cause disease in humans and animals. Front Biosci J Virtual Libr 13:6938–6946. https://doi.org/10.2741/3200
11. De Rouck S, İnak E, Dermauw W, Van Leeuwen T (2023) A review of the molecular mechanisms of acaricide resistance in mites and ticks. Insect Biochem Mol Biol 159:103981. https://doi.org/10.1016/j.ibmb.2023.103981
12. Dolenga CJR, Anjos A, Barbosa VHG, et al (2022) Acaricidal effect of major compounds to control *Rhipicephalus microplus* (Canestrini, 1887) in dairy cows and possible alternatives for reversing multidrug resistance. Rev Bras Parasitol Veterinária 31:e005422. https://doi.org/10.1590/S1984-29612022028
13. Domingues LN, Brasil BSAF, Bello ACPP, et al (2012) Survey of pyrethroid and organophosphate resistance in Brazilian field populations of *Rhipicephalus* (*Boophilus*) *microplus*: detection of C190A mutation in domain II of the para-type sodium channel gene. Vet Parasitol 189:327–332. https://doi.org/10.1016/j.vetpar.2012.04.031
14. Drummond RO, Ernst SE, Trevino JL, et al (1973) *Boophilus annulatus* and *B. microplus*: laboratory tests of insecticides. J Econ Entomol 66:130–133. https://doi.org/10.1093/jee/66.1.130
15. Elango G, Rahuman AA (2011) Evaluation of medicinal plant extracts against ticks and fluke. Parasitol Res 108:513–519. https://doi.org/10.1007/s00436-010-2090-9
16. Faza AP, Pinto ISB, Fonseca I, et al (2013) A new approach to characterization of the resistance of populations of *Rhipicephalus microplus* (Acari: Ixodidae) to organophosphate and pyrethroid in the state of Minas Gerais, Brazil. Exp Parasitol 134:519–523. https://doi.org/10.1016/j.exppara.2013.04.006
17. Fernández-Salas A, Alonso-Díaz MA, Acosta-Rodríguez R, et al (2011) In vitro acaricidal effect of tannin-rich plants against the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae). Vet Parasitol 175:113–118. https://doi.org/10.1016/j.vetpar.2010.09.016
18. George JE, Pound JM, Davey RB (2004) Chemical control of ticks on cattle and the resistance of these parasites to acaricides. Parasitology 129 Suppl:S353-366. https://doi.org/10.1017/s0031182003004682
19. Ghosh S, Tiwari SS, Kumar B, et al (2015) Identification of potential plant extracts for anti-tick activity against acaricide resistant cattle ticks, *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae). Exp Appl Acarol 66:159–171. https://doi.org/10.1007/s10493-015-9890-7
20. Gobbo-Neto L, Lopes NP (2007) Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. Quím Nova 30:374–381. https://doi.org/10.1590/S0100-40422007000200026
21. Grisi L, Leite RC, Martins JRDS, et al (2014) Reassessment of the potential economic impact of cattle parasites in Brazil. Rev Bras Parasitol Veterinária 23:150–156. https://doi.org/10.1590/S1984-29612014042

22. Higa LDOS, Garcia MV, Barros JC, et al (2015) Acaricide Resistance Status of the Rhipicephalus microplus in Brazil: A Literature Overview. Med Chem 5:. https://doi.org/10.4172/2161-0444.1000281
23. Higa LDOS, Garcia MV, Barros JC, et al (2016) Evaluation of *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) resistance to different acaricide formulations using samples from Brazilian properties. Rev Bras Parasitol Veterinária 25:163–171. https://doi.org/10.1590/S1984-29612016026
24. Jonsson NN (2006) The productivity effects of cattle tick (*Boophilus microplus*) infestation on cattle, with particular reference to Bos indicus cattle and their crosses. Vet Parasitol 137:1–10. https://doi.org/10.1016/j.vetpar.2006.01.010
25. Khan A, Nasreen N, Niaz S, et al (2019) Acaricidal efficacy of *Calotropis procera* (Asclepiadaceae) and *Taraxacum officinale* (Asteraceae) against *Rhipicephalus microplus* from Mardan, Pakistan. Exp Appl Acarol 78:595–608. https://doi.org/10.1007/s10493-019-00406-z
26. Klafke G, Webster A, Dall Agnol B, et al (2017) Multiple resistance to acaricides in field populations of *Rhipicephalus microplus* from Rio Grande do Sul state, Southern Brazil. Ticks Tick-Borne Dis 8:73–80. https://doi.org/10.1016/j.ttbdis.2016.09.019
27. Laing G, Aragrande M, Canali M, et al (2018) Control of Cattle Ticks and Tick-Borne Diseases by Acaricide in Southern Province of Zambia: A Retrospective Evaluation of Animal Health Measures According to Current One Health Concepts. Front Public Health 6:45. https://doi.org/10.3389/fpubh.2018.00045
28. Lázaro SF, Fonseca LD, Fernandes RC, et al (2012) Efeito do extrato aquoso do algodão de seda (*Calotropis procera* Aiton) sobre a eficiência reprodutiva do carrapato bovino. Rev Bras Plantas Med 14:302–305. https://doi.org/10.1590/S1516-05722012000200008
29. Linhares LPMB, Pereira BVN, Dantas MKG, et al (2022) *Schinopsis brasiliensis* Engler—Phytochemical Properties, Biological Activities, and Ethnomedicinal Use: A Scoping Review. Pharmaceuticals 15:1028. https://doi.org/10.3390/ph15081028
30. Lorenzi H (2002) Brazilian Trees, Volume 1: A Guide to the Identification and Cultivation of Brazilian Native Trees, 4th edn. Instituto Plantarum, Nova Odessa
31. Madzimure J, Nyahangare ET, Hamudikuwanda H, et al (2011) Acaricidal efficacy against cattle ticks and acute oral toxicity of *Lippia javanica* (Burm F.) Spreng. Trop Anim Health Prod 43:481–489. https://doi.org/10.1007/s11250-010-9720-1
32. Maikai VA, Maikai BV, Kobo PI (2009) Antimicrobial Properties of Stem Bark Extracts of *Ximenia americana*. J Agric Sci 1:p30. https://doi.org/10.5539/jas.v1n2p30
33. Maina GJ, Timothy M, Muchunu MJ (2018) Antifleas Activity and Safety of *Tithonia diversifolia* and *Senna didymobotrya* Extracts. J Pharm Pharmacol Res 02: https://doi.org/10.26502/jppr.0012
34. Morais HLMDN, Feitosa TC, Rodrigues JGM, et al (2020) Hydroalcoholic extract of *Caryocar brasiliense* Cambess. leaves affect the development of *Aedes aegypti* mosquitoes. Rev Soc Bras Med Trop 53:e20200176. https://doi.org/10.1590/0037-8682-0176-2020
35. Morais-Costa F, Soares ACM, Bastos GA, et al (2015) Plants of the Cerrado naturally selected by grazing sheep may have potential for inhibiting development of *Haemonchus contortus* larva. Trop Anim Health Prod 47:1321–1328. https://doi.org/10.1007/s11250-015-0866-8
36. Pereira DFS, Ribeiro HS, Gonçalves AAM, et al (2022) *Rhipicephalus microplus*: An overview of vaccine antigens against the cattle tick. Ticks Tick-Borne Dis 13:101828. https://doi.org/10.1016/j.ttbdis.2021.101828

37. Quadros DG, Johnson TL, Whitney TR, et al (2020) Plant-Derived Natural Compounds for Tick Pest Control in Livestock and Wildlife: Pragmatism or Utopia? Insects 11:490. https://doi.org/10.3390/insects11080490
38. Rajakumar G, Rahuman AA, Roopan SM, et al (2015) Efficacy of larvicidal activity of green synthesized titanium dioxide nanoparticles using *Mangifera indica* extract against blood-feeding parasites. Parasitol Res 114:571–581. https://doi.org/10.1007/s00436-014-4219-8
39. Rodrigues DS, Leite RC (2013) Economic impact of *Rhipicephalus* (*Boophilus*) *microplus*: estimate of decreased milk production on a dairy farm. Arq Bras Med Veterinária E Zootec 65:1570–1572. https://doi.org/10.1590/S0102-09352013000500039
40. Santos CCS, Araújo SS, Santos ALLM, et al (2014) Evaluation of the toxicity and molluscicidal and larvicidal activities of *Schinopsis brasiliensis* stem bark extract and its fractions. Rev Bras Farmacogn 24:298–303. https://doi.org/10.1016/j.bjp.2014.07.006
41. Shyma KP, Gupta JP, Ghosh S, et al (2014) Acaricidal effect of herbal extracts against cattle tick *Rhipicephalus* (*Boophilus*) *microplus* using in vitro studies. Parasitol Res 113:1919–1926. https://doi.org/10.1007/s00436-014-3839-3
42. Silva junior MC (2012) 100 Árvores do Cerrado - Sentido Restrito: Guia de Campo, 1st edn. Rede de Sementes do Cerrado
43. Vasconcelos VO, Costa EGL, Moreira VR, et al (2018) Efficacy of plants extracts from the Cerrado against adult female of *Dermacentor nitens* (Acari: Ixodidae). Exp Appl Acarol 75:419–427. https://doi.org/10.1007/s10493-018-0276-5
44. Wadhwani BD, Mali D, Vyas P, et al (2021) A review on phytochemical constituents and pharmacological potential of *Calotropis procera*. RSC Adv 11:35854–35878. https://doi.org/10.1039/D1RA06703F
45. Wanzala W (2017) Potential of Traditional Knowledge of Plants in the Management of Arthropods in Livestock Industry with Focus on (Acari) Ticks. Evid-Based Complement Altern Med ECAM 2017:8647919. https://doi.org/10.1155/2017/8647919
46. Welsh JA, Braun H, Brown N, et al (2019) Production-related contaminants (pesticides, antibiotics and hormones) in organic and conventionally produced milk samples sold in the USA. Public Health Nutr 22:2972–2980. https://doi.org/10.1017/S136898001900106X

Tables

Table 1 Plant species collected at the municipally of Montes Claros, Brazil and the preparations used for the experiments.

Plants	Vernacular name	Family	Voucher	Preparations tested[a]
Native species				
Piptadenia viridiflora Kunth	Surucucu	*Fabacea*	2283	EE-EA
Annona crassiflora Mart.	Panã	*Annonaceae*	3475	EE
Caryocar brasiliense Camb.	Pequi	*Caryocaraceae*	338	EE
Ximenia americana L.	Ameixa do Cerrado	*Olacaceae*	211	EE-EA
Schinopsis brasilienses Engl.	Brauna	*Anacardiaceae*	377	EE
Exotic species				
Mangifera indica L.	Manga	*Anacardiaceae*	-	EE
Tithonia diversifolia (Hemsl.)	Girassol Mexicano	*Asteraceae*	-	EA
Calotropis procera (Aiton)	Algodão de Seda	*Apocynacea*	-	Latex - EA

[a]Aquous extract (EA); Ethanolic extracts (EE).

Table 2 Sensitivity of different field populations and strains of *Rhipicephalus microplus* to cypermethrin by the larval packet test.

Populations/strains	% mortality at DD[a]	Resistance level[b]	LC_{50}[c]	(CI95%)[d]	RR[e] at LC50	LC_{90}	(CI95%)	RR at LC90
Porto Alegre	99.0	S	0.00014	0.0003-0.0001	nc[f]	0.070	0.04-0.096	nc
Jaguar	2.8	RIV	13.9	10.2-20.2	9928.6	491.3	254-1122	70185
Bocaiuva	88.5	RI	0.09	0.08-0.11	64.3	1.04	0.89-1.2	77.5
Santa Rita	66.3	RII	0.02	0.01-0.03	14.3	5.4	3.57-9.38	14.8
Igarapé	54.7	RIII	0.13	0.12-0.15	92.8	1.3	1.1-1.6	19.4
Nova Esperança	14.3	RIV	0.44	0.39-0.49	314.3	4.5	3.7-5.5	64.71
Moema	10.6	RIV	nc	nc	nc	nc	nc	nc
Funilândia	10.1	RIV	4834	593- nc	34530	nc	nc	nc
Boa Vitória	3.2	RIV	1056	273-9121	754692	Nc	nc	nc

[a]DD: discriminating dose (Cypermethrin 0.2%); [b]Resistance levels based on the % mortality at DD according to Klafke et al. (2017), S- sensible, R1 to RIV - resistance level I to IV; [c]Values represented as % of active ingredient; [d]CI: confidence interval; [e]RR: resistance ratio; [f]nc: values not calculated.

Table 3 Sensitivity of different field populations and strains of *Rhipicephalus microplus* to triclorfon by the larval packet test.

Populations/strains	% mortality at DD[b]	Resistance level[c]	LC_{50}[d]	(CI95%)[e]	RR[f] at LC50	LC_{90}	(CI95%)	RR at LC90
Porto Alegre	99.68	S	0.16	0.15-0.17	nc[g]	0.55	0.50-0.63	nc
Jaguar	19.18	RIV	48.07	29.43-90.9	300.4	30.887.1	8.680.1-	56.1
Bocaiuva	94.72	RI	0.06	0.05-0.07	0.37	0.51	0.45-0.59	0.9
Santa Rita	91.12	RI	0.07	0.06-0.09	0.43	0.81	0.7-0.9	1.4
Igarapé	81.22	RII	0.17	0.15- 0.2	1.06	4.45	3.7-5.4	8.09
Nova Esperança	77.99	RII	0.23	0.19-0.26	1.43	4.53	3.8-5.4	8.2
Moema	40.92	RIII	1.28	1.07-1.5	8.0	281.8	161.2-558.6	512.3
Funilândia	34.85	RIII	2.92	2.4-3.4	18.2	209.9	134.9-354.9	381.6
Boa Vitória	25.47	RIII	14.81	11.4-20.2	92.56	1.312.2	680.4-2945	2.385.4

[a]Neguvon®, Bayer; [b]DD: discriminating dose (triclorfon 0,99%); [c]Resistance levels based on the % mortality at DD according to Klafke et al. (2017), S- sensible, R1 to RIV - resistance level I to IV; [d]Values represented as % of active ingredient; [e]CI: confidence interval; [f]RR: resistance ratio; [g]nc: values not calculated.

Table 4 Mortality of *Rhipicephalus microplus* larvae (Boa Vitoria, resistant population) treated with the extracts of plants from the Brazilian savanna.

Treatment (mg mL^{-1})	*Annona crassiflora*	*Caryocar brasiliense*	*Pitadenia viridiflora*		*Schinopsis brasiliensis*	*Ximenia americana*	
	EE[a]	EE	AE[b]	EE	EE	AE	EE
100.0	8.57 a	32.93 a	5.21 a	10.12 a	25.92 a	10.23 a	25.00 a
50.0	6.97 a	27.14 a	2.00 b	9.44 a	18.40 b	7.25 a	25.76 a
25.0	5.54 a	26.97 b	0.00 c	8.37 a	11.24 c	4.79 b	16.94 a
12.5	4.98 a	23.92 b	0.00 c	6.81 b	14.39 c	4.22 b	12.83 b
6.25	3.37 b	31.50 a	0.00 c	6.79 b	12.08 c	2.01 c	5.03 b
3.12	0.78 c	23.40 b	0.00 c	5.65 b	19.11 b	1.80 c	6.42 b
1.56	1.25 c	24.99 b	0.00 c	2.59 c	10.95 c	0.98 c	10.63 b
0.78	0.64 c	25.02 b	0.00 c	0.50 d	7.77 d	0.00 c	1.36 b
Distilled water	3.55 b	3.55 c	3.55 b	1.09 d	2.23 e	1.09 c	3.55 b
Cypermethrin[c]	3.20 b	3.20 c	3.20 b	4.32 c	4.93 d	4.32 b	3.20 b
CV(%)[d]	4.95	2.06	9.84	6.72	3.62	5.56	7.29

[a]Ethanolic extracts (EE); [b]Aquous extracts (AE); [c]aCypermethrin 0,2%; [d]CV: coefficient of variation. Different letters in the same column indicate statistically difference (p<0.05; Scott-Knott test).

Table 5 Mortality of *Rhipicephalus microplus* larvae (Boa Vitoria, resistant population) treated with the extracts of exotic plants from the Brazilian savanna.

Treatment (mg mL^{-1})	*Mangifera indica* EE[a]	*Tithonia diversifolia* AE[b]	*Calotropis procera* AE	*Calotropis procera* Latex
100.0	5.57 a	8.00 b	44.90 a	62.92 a
50.0	5.22 a	7.23 b	16.77 b	51.21 b
25.0	5.25 a	5.60 c	15.79 b	49.14 b
12.5	5.80 a	5.59 c	10.25 c	41.24 c
6.25	5.67 a	3.84 d	16.47 b	31.77 d
3.12	5.77 a	3.35 d	12.09 b	22.94 e
1.56	0.71 c	0.00 e	16.94 b	15.46 f
0.78	0.72 c	0.00 e	9.98 c	5.44 g
Distilled water	1.09 c	3.55 d	3.81 c	2.88 g
Cypermethrin [c]	4.32 b	11.20 a	4.93 c	3.42 g
CV(%)[d]	6.72	7.82	24.51	26.05

[a]Ethanolic extracts (EE); [b]Aquous extracts (AE); [c]aCypermethrin 0,2%; [d]CV: coefficient of variation. Different letters in the same column indicate statistically difference ($p<0.05$; Scott-Knott test).

Table 6 Reproductive efficiency of adult female *Rhipicephalus microplus* (Boa Vitoria, resistant population) treated with aqueous extracts of *Calotropis procera* and ethanolic extracts of *Schinopsis brasiliensis* and *Ximenia americana*.

Treatments	*Calotropis procera*			*Schinopsis brasiliensis*			*Ximenia americana*		
(mg mL^{-1})	ELC[a]	H[b](%)	E[c](%)	ELC[a]	H[b](%)	E[c](%)	ELC[a]	H[b](%)	E[c](%)
100.0	23.45 c	38.00 b	73.20 c	38.25 b	65.60 b	34.0 b	22.46 c	64.09 b	60.79 a
50.0	30.04 b	12.10 c	89.60 b	38.91 b	85.80 a	10.8 d	41.86 a	86.91 ab	3.35 b
25.0	19.89 d	6.70 d	96.80 a	32.13 c	82.60 a	29.0 c	38.63 ab	92.53 a	3.93 b
Cypermethrin[d]	25.20 c	30.00 b	79.53 c	25.20 d	30.00 c	79.53 a	25.20 c	30.00 c	79.53 a
Distilled water	42.92 a	85.66 a	-	42.92 a	85.66 a	-	42.92 a	85.66 ab	-
CV (%)[e]	29.60	22.56	38.50	14.30	19.50	62.50	36.50	16.60	23.58

[a]ELC: Egg laying capacity = (weight of egg mass/initial weight of females) × 100; [b]H: Larval hatching; [c]E: Efficiency = (Control RE − Product RE / Control RE) × 100; [d]aCypermethrin 0,2%; [e]CV: Coefficient of variation. Different letters in the same column indicate statistically difference ($p<0.05$; Duncan test).