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The potential of *Acmella oleracea* (Jambu) extract in the control of semi-engorged *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) female ticks

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ABSTRACT

The present study evaluated the efficacy of *Acmella oleracea* extract, and the susceptibility of semi-engorged *R. sanguineus* females exposed to different concentrations of the extract, with lethal concentration 50 % (LC₅₀) and confidence interval 95%. The ticks were mounted on Petri dishes and immersed in the different concentrations of the extract, distilled water or ethanol 50% + DMSO 1% for 5 minutes, dried and kept in BOD incubator for 7 days. The results showed the daily mortality rate among semi-engorged *R. sanguineus* females at different concentrations of the extract. The mortality data obtained in bioassay 2 were subjected to Probit analysis, through which a LC₅₀ of 24,883 mg/ml (limits: 22,331 to 28,003 mg/ml) was determined, at a 95% confidence interval. Thus, this study demonstrated the sensitivity and susceptibility of semi-engorged *R. sanguineus* females to *Acmella oleracea* extract. The acaricidal potential was effective as from the application of the extract at the concentration of 12.5 mg/ml and was dose-dependent. i.e., higher mortality rates were observed as the concentration increased, with LC₅₀ of 24,883 mg/ml reaching 100% at the concentration of 100 mg/ml in 24h.

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Extract; *Rhipicephalus sanguineus*; tick; natural compound; potential

1. Introduction

Rhipicephalus sanguineus Latreille, 1806 (Acari, Ixodidae) ticks, with widespread distribution in Brazil and across the globe, have considerable importance for human and animal health. These ectoparasites are pathogen vectors for the hosts, also affecting human populations (Demma et al. 2005; Dantas-Torres 2008, 2010; Ereemeeva et al. 2011).

R. sanguineus control is mostly performed through the application of chemical acaricides to the environment and/or directly to the dogs (Labruna and Pereira 2001; Dantas-Torres et al. 2006; de Oliveira et al. 2008, 2009). However, these substances, which are frequently misused, and their indiscriminate application, lead to the selection of resistant populations (Labarthe 1994; Leal et al. 2003). The literature has reported *R. sanguineus* resistance to acaricides commercialized around the globe (Miller et al. 2001; Burridge et al. 2003; Estrada-Pena 2005; Miranda et al. 2007).

Considering the risks resulting from environmental contamination caused by indiscriminate use of chemical products and the resistance developed by ectoparasites, natural compounds, i.e. extracts from plants, with acaricidal potential emerge as a highly promising control strategy (Miranda et al. 2007; Vendramini et al. 2012).

In this sense, plants from the family Meliaceae, including the species *Melia* (Oelrichs et al. 1983), *Trichilia*, *Toona*, *Aglaia* and *Azadirachta indica* (Martinez 2002), among others, have been proven to be efficient against Arthropoda. Additionally, these substances degrade quickly, once they are obtained from renewable sources (Martinez 2002).

Acmella oleracea, popularly known as Jambu, is a plant of the family Asteraceae, typical from the north region of the Brazil, with hot and humid climate. It is used as condiment, as an ingredient in cosmetic composition and in popular medicine, with bactericidal (Pessini et al. 2003), fungistatic and fungicidal (Fabry et al. 1996) properties, in addition to

presenting ovicidal and larvicidal activity against *Aedes aegypti* (dengue), *Anopheles culicifacies* (malaria) and *Culex quiquefasciatus* (malaria) (Saraf and Dixit 2002; Simas et al. 2013) mosquitoes. Recent studies have demonstrated the acaricidal properties of the extracts obtained from *A. oleracea* against *Rhipicephalus B. microplus*, confirming its high efficiency against larvae and engorged females (Castro et al. 2014).

Acmella oleracea is a small, semi-erect herbaceous plant, measuring 30–60 cm tall, with cylindrical fleshy stem and decumbent branches. The primary root has axial growth, with abundant lateral and adventitious roots in the stems and branches in contact with the soil. Its leaves are simple, with broadly ovate blade, and sparse hair on both surface. The flowers are small and yellow, arranged in globose chapters, with approximately 1 cm of diameter. The fruit is a very small achene with dark grey pericarp, partially surrounded by membranous paleas (Favoreto and Gilbert 2010). The plant blossoms all year long in the tropics and in early summer in temperate regions (Hind and Biggs 2003).

The main biological effects of *Acmella oleracea* are attributed to the spilanthal or affinin [N-2-Methylpropyl]-2,6,8-decatrienamide or N-isobutyl-2E,6Z,8E-decatrienamide], an aliphatic alkamide with molecular formula C₁₄H₂₃NO, described as a yellowish viscous oil, obtained for the first time by Gerber in 1903 (Ramsewak 1999).

The evaluation of the efficacy of a chemical is usually performed through *in vitro* bioassays, which are relatively simple, inexpensive and require little equipment (Scott 1995).

Thus, the present study aimed to verify the efficacy of *Acmella oleracea* extract and the level of susceptibility of semi-engorged *R. sanguineus* adult females exposed to different concentrations through *in vitro* bioassay protocol (AIT), monitored on a daily basis, determining the LC₅₀ (lethal concentration 50%) with a 95% confidence interval.

2. Material and methods

2.1. Study site

The present study was conducted in the facilities of the Histology Laboratory of the Biology Department I.B. – UNESP – Campus Rio Claro, SP, Brazil. The tick colonies were kept in this institution by the Brazilian Centre of Studies on Ticks Morphology (BCSTM), under the coordination of Prof. Dr. Maria Izabel Camargo Mathias.

2.2. Chemical substance

2.2.1. Natural extract of *Acmella oleracea*

The crude ethanolic extract from the aerial parts (flowers, leaves and stem) of *A. oleracea* was provided by the Chemical Laboratory of Natural Products from the Pluridisciplinary Center of Chemical, Biological and Agricultural Research (CPQBA) University of Campinas (UNICAMP), Campinas, SP, Brazil, under the supervision of Dr. Rodney Alexandre Ferreira Rodrigues and support from postgraduate student Lais Thiemi Yamane.

The plants were cultivated in the experimental field of CPQBA/UNICAMP, located in Paulinia, SP, Brazil (geographical coordinates $-22^{\circ} 47' 52''$ S, $-47^{\circ} 6' 49''$ W). The seeds were provided by the company Centroflora (Botucatu, SP, Brazil) and the aerial parts – flowers, leaves and stem – were collected in April 2015. The plant was identified by Dr. John F. Pruski of Missouri Botanical Garden (USA), and a voucher specimen was deposited in CPQBA/UNICAMP Herbarium, Campinas, SP, Brazil, number 181,452. License for genetic testing (CGEN) number is 010577/2014-9.

2.2.2. Drying and milling the plant material

After collected, the aerial parts (flowers, leaves and stems) of *A. oleracea* were dried at 40°C under forced ventilation for 48 h until constant weight (Rodrigues et al. 2006), milled with a knife mill and passed through a 48-mesh sieve – 0.297 mm. The resulting material was placed in polypropylene-coated Kraft bags and kept in freezer at -20°C .

2.2.3. Preparing the crude ethanolic extract of *Acmella oleracea* (L.) R. L. Jansen

The extraction was performed through mechanical stirring in a stainless tank, at room temperature, using ethanol 96° GL (proportion 1:5 plant/solvent) for 1.5 h. The material was filtered three times to separate the residues (Rodrigues et al. 2006). The resulting crude extract was filtered, homogenized and concentrated under vacuum in rotary evaporator at 40°C , lyophilized until a constant weight was attained, stored in amber flasks and kept in freezer until use.

2.2.4. Analytical monitoring of spilanthol

The quantification of spilanthol in the extract was performed using a gas chromatograph coupled with a mass detector (GC–MS, Agilent® 5890 Series II mass selective detector Agilent® 5970 EI 70 eV) equipped with a fused silica column WCOT, HP5–MS, Agilent®, dimensions 30 m $\times$ 0.25 mm $\times$ 0.25 μm . The analysis conditions were: injector temperature: 220°C ; detector temperature: 250°C , temperature programme: $60\text{--}240^{\circ}\text{C}$ ($3^{\circ}\text{C}/\text{min}$), sample injection using split mode at 1:40 ratio, Helium gas was used as carrier at 0.7 bar, 1 mL/min.

2.3. *Rhipicephalus sanguineus* ticks (Latreille, 1806)

R. sanguineus semi-engorged females, weighing 27 mg on average (about 5 days of feeding) were used throughout the experiment. The ticks were provided by the Animal Facility of the Department of Biology – UNESP, Rio Claro campus/São Paulo, Brazil, where the colony is maintained under controlled conditions (28°C , 85% humidity, and 12-h photoperiod) in a Biological Oxygen Demand (BOD) incubator. Unfed *R. sanguineus* couples (25 couple/

infestation) were allowed to feed on naive New Zealand white rabbits following Bechara et al. (1995) until reaching the semi-engorged stage. The semi-engorged stage of the females was chosen due to the high parasitic efficiency in this phase.

2.4. Hosts

New Zealand white rabbits, weighing between 3 and 3.5 kg, were used as hosts. Rabbits were obtained from the Animal Facility of UNESP – Campus Botucatu/São Paulo – Brazil and housed in the Animal Facility of UNESP – Rio Claro Campus/São Paulo – Brazil. The animals had not had prior contact with ticks or acaricides, and were kept under controlled conditions. During the entire experiment, the rabbits were maintained in cages, receiving water and commercial food *ad libitum*.

This study was approved by the Ethics Committee for Animal Experimentation of UNESP/SP/Brazil, protocol no. 6334/2014.

2.5. Extract concentration

The initial concentration of the extract was established according to Castro et al. (2014). The concentrations used in bioassay 1 (1.6 mg/ml, 3.1 mg/ml, 6.2 mg/ml, 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml) were obtained by diluting the extract in a solution of 50% ethanol + 1% DMSO. All concentrations were stored and labelled in Petri dishes until use. Each treatment was performed in duplicate.

After determining the mortality interval (12.5–50 mg/ml) in bioassay 1, new dilutions were performed in order to obtain other concentrations within this mortality interval: 14.3 mg/ml, 16.6 mg/ml, 20 mg/ml, 25 mg/ml, 33.3 mg/ml and 40 mg/ml. These concentrations were used in bioassay 2.

2.6. Bioassay 1

Twenty semi-engorged *R. sanguineus* females (10 ticks per Petri dish) were used for each concentration of extract and control groups 1 and 2. The ticks were rinsed in a sieve under running tap water, dried with soft paper towel, mounted on labelled Petri dishes and immersed in each concentration for 5 min. For control groups 1 and 2, the females were immersed in distilled water and in a solution of 50% ethanol and 1% DMSO for 5 min, respectively. Then, the ticks were dried, mounted on Petri dishes and kept in BOD incubator for 7 days, because acaricides usually act slowly in the physiology of ticks, and their effects are only visible after this period Roma et al. (2009).

Mortality rates and the effects of extract were monitored daily. The mortality interval was determined by observing the behaviour of ticks up to the 7th day of treatment. Ticks were considered dead when not moving their legs when touched with the tip of a paintbrush.

2.7. Bioassay 2

The *R. sanguineus* females were rinsed in a sieve under running tap water and dried with soft absorbent paper. The immersion procedure is the same described for bioassay 1. One hundred and sixty semi-engorged females (eight treatment groups with 20 females each – 10 individuals on each Petri dish) were mounted on labelled Petri dishes and immersed in each concentration of the extract for 5 min.

The control group 1 and 2 ticks were immersed in distilled water and in 50% ethanol and 1% DMSO for the same period. Ticks were dried in absorbent paper, mounted on labelled Petri dishes and kept in BOD incubator ($28 \pm 1^{\circ}\text{C}$, 80% humidity and 12 h photoperiod) for 7 days.

Posteriorly, the level of susceptibility of the females to each concentration of the bioassay 2 was analysed, and daily mortality for each concentration was monitored. Mortality criterion was the inability to move the locomotor appendages.

Table 1. Percentage of dead of semi-engorged females *Rhipicephalus sanguineus* ticks exposed to different concentrations of the extract of *Acmella oleracea* – Bioassay 1.

		Percentage of dead ticks/days of treatment						
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Concentration of <i>Acmella oleracea</i> (mg/ml)	Control 1	0	0	0	0	0	0	0
	Control 2	0	0	0	0	0	0	0
	1.6 mg/ml	0	0	0	0	0	0	0
	3.1 mg/ml	0	0	0	0	0	0	0
	6.2 mg/ml	0	0	0	0	0	0	0
	12.5 mg/ml	0	0	0	0	5	5	10
	25 mg/ml	10	15	20	20	30	40	50
	50 mg/ml	15	20	25	35	55	60	90
	100 mg/ml	100	100	100	100	100	100	100

Table 2. Percentage of dead of semi-engorged females *Rhipicephalus sanguineus* ticks exposed to different concentrations of the extract of *Acmella oleracea* – Bioassay 2.

		Percentage of dead ticks/days of treatment						
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Concentration of <i>Acmella oleracea</i> (mg/ml)	Control 1	0	0	0	0	0	0	0
	Control 2	0	0	0	0	0	0	0
	14.3 mg/ml	0	0	0	0	0	0	5
	16.6 mg/ml	0	0	0	0	5	10	20
	20 mg/ml	0	5	10	10	15	30	50
	25 mg/ml	15	15	15	15	35	35	55
	33.3 mg/ml	5	10	15	15	35	40	65
	40 mg/ml	10	20	30	30	40	65	80

2.8. Statistical analysis

The mortality data for the semi-engorged *R. sanguineus* females in bioassay 2 were analysed through one-way analysis of variance (ANOVA) with the application of *post hoc* Tukey's test, significance levels set at $p < 0.05$, using the software Graph-Pad Prism v.6 (GraphPad Software Inc., San Diego, CA, EUA). Lethal concentration (LC_{50}) and 95% confidence interval were calculated through Probit analysis using software BioStat v5.

3. Results

The efficacy of the extract from *Acmella oleracea* against semi-engorged *R. sanguineus* females was verified using Drummond et al. (1973) test. In the present study, the results were obtained with the application of 13 different concentrations of this extract, tested in duplicate (Tables 1–3 and Figure 1).

Bioassay 1 showed that the semi-engorged *R. sanguineus* females were not affected by all the extract concentrations used in this study (Table 1).

No mortality was observed following treatment with lower concentrations of the extract (1.6 mg/ml, 3.1 mg/ml and 6.2 mg/ml) throughout the 7-day observation period (Table 1). No morphological or behavioural alterations were recorded for the individuals treated with these concentrations.

At the concentration of 12.5 mg/ml, the extract caused immediate reactions, causing alterations on the living individuals or even death. On the first, second, third and fourth days following treatment, the locomotor capacity decreased. Dead ticks were found 5 days following treatment (Table 1).

At higher concentrations (25 mg/ml, 50 mg/ml and 100 mg/ml), a high mortality rate is observed since the first day of treatment. In the concentrations of 25 mg/ml and 50 mg/ml, the still-alive individuals displayed behavioural alterations, such as progressive decrease in locomotor activity, coordination loss, prostration in inverted position (upside down), stretching of all legs and paralysis. At the highest concentration (100 mg/mL), the mortality rate was 100%, observed as early as 24 h of exposure (Table 1).

At concentrations, equal or higher than 12.5 mg/ml, a gradual increase in mortality (5–100%) was observed as the extract concentration increased (12.5 mg/ml, 25 mg/ml, 50 mg/ml and

100 mg/ml) (Figure 1). Likewise, the effects of the extract progressively increased over the observation period. The concentration of 50 mg/ml caused mortality of 15%, 25%, 55% and 90% on the first, third, fifth and seventh day, respectively (Table 1).

Two mortality peaks (fifth and seventh days) were observed for the females subjected to higher concentrations in bioassay 1 (12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml) (Table 1).

The mortality data from bioassay 1, i.e. the number of dead semi-engorged *R. sanguineus* females at each concentration, determined the mortality interval of 12.5 mg/ml at 50 mg/ml of extract. Based on this data, new dilutions were performed and the concentrations to be used in bioassay 2 were obtained (Table 2).

The *R. sanguineus* females were sensitive to the extract concentrations tested in bioassay 2 (14.3 mg/ml, 16.6 mg/ml, 20 mg/ml, 25 mg/ml, 33 mg/ml and 40 mg/ml). At lower concentrations (14.3 mg/ml and 16.6 mg/ml), the mortality rate ranged between 5% and 20%. The concentrations of 20 mg/ml and 25 mg/ml showed 50% and 55% of dead individuals, respectively; while in the higher concentrations (33.3 mg/ml and 40 mg/ml) mortality rates ranged from 60% to 80%. These data demonstrated that the mortality rates (5–80%) gradually increased in higher concentrations of the extract (14.3 mg/ml, 16.6 mg/ml, 20 mg/ml, 25 mg/ml, 33 mg/ml and 40 mg/ml) (Figure 1, Table 2).

Moreover, in bioassay 2, the extract caused the highest mortality peaks on the fifth, sixth and seventh days of the experiment (Table 2).

All the extract concentrations (14.3 mg/ml, 16.6 mg/ml, 20 mg/ml, 25 mg/ml, 33 mg/ml and 40 mg/ml) used in bioassay 2 interfered in the behaviour of the individuals tested. Over the 7-day period, the ticks presented progressive decrease in locomotor

Table 3. Results of the Probit analysis based on the mortality of semi-engorged females *R. sanguineus* ticks exposed to the extract of *Acmella oleracea* in Bioassay 2.

LC_{50}	24.883. limits: 22.331–28.003
Standard error	0.0283
Degrees of freedom	4
Heterogeneity	0.05
Estimation of the confidence interval g (0.95)	0.1368

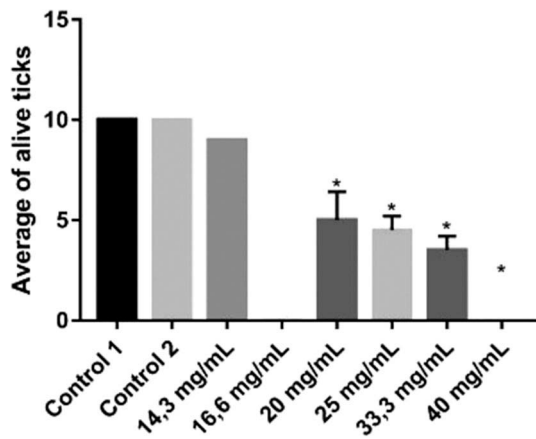


Figure 1. Bioassay 2: Average of live *R. sanguineus* ticks on the 7th day of exposure to different *Acmeilla oleracea* extract concentrations. *indicates statistically significant difference ($p < 0.05$). Control 1 = distilled water; Control 2 = 50% ethanol and 1% DMSO.

capacity, loss of coordination, prostration in inverted position (upside down), stretching of all legs and paralysis.

No morphological alterations were recorded following treatment with lower extract concentrations (1.6 mg/ml, 3.1 mg/ml and 6.2 mg/ml). However, the semi-engorged *R. sanguineus* exposed to the extract at the concentration of 12.5 mg/ml presented integument dehydration, and this condition evolved as the extract concentration increased (Table 2).

No statistically significant mortality was observed in the individuals belonging to control groups 1 and 2, neither behavioural alterations, nor abnormalities in the morphology, coloration and consistency of the integument over the observation period (7 days) (Figure 1).

Mortality data from bioassay 2 were subjected to Probit analysis to estimate LC_{50} at 95% confidence interval, establishing $g(95)$: $LC_{50} = 24.883$ mg/ml (limits: 22.331–28.003) (Table 3).

4. Discussion

Ticks have significant economic importance as ectoparasites and pathogen vectors worldwide, representing one of the biggest obstacles to cattle rearing, causing leather damage, weight loss and reduced milk production. In addition, these ectoparasites are responsible for considerable economic losses associated with medications and medical-veterinary assistance (Walker et al. 2000; Sonenshine and Roe 2014).

The main method to control these ectoparasites is the use of chemical acaricides, which, despite their efficiency, result in the development of resistant populations, in addition to offering risks to other animals and human beings due to the contamination of the environment through the accumulation of toxic residues (Chagas 2004; Dantas-Torres et al. 2006). Therefore, the search for less aggressive and sustainable acaricides is of the utmost importance (de Oliveira et al. 2012).

In this sense, a promising alternative is the use of extracts from plants with acaricidal properties. Several plant species have had their efficacy against ticks tested and the literature reports the success of extracts and other plant substances in controlling different tick species in distinct developmental stages (Chagas 2004).

In studies using *R. B. microplus*, the essential oils *Eucalyptus citriodora* and *Eucalyptus staigeriana* caused 100% larval mortality at the concentration of 10% (Chagas et al. 2002). The aqueous extract of *Simarouba versicolor* inhibited 100% oviposition at the concentrations of 1.72% (Pires et al. 2007); and the extract from the roots of *S. versicolor* showed a 75% acaricidal activity at the concentration of 2.5% (Catto et al. 2009). Extracts of *S. australis*

leaves applied to *R. B. microplus* females showed efficacy level of 34% and 66% when diluted in water and ethanol, respectively (Krawczak et al. 2011).

Considering this information and the fact that *R. sanguineus* ticks have become an important pest, the present study tested the sensitivity and susceptibility of semi-engorged *Rhipicephalus sanguineus* adult females exposed to different concentrations of *Acmeilla oleracea* extract, analysing the efficacy of the extract to control females from this important species.

The research methodology consisted of an *in vitro* test, where the engorged females were immersed in different formulations with posterior observation and comparison with a control group (Drummond et al. 1973). This is an efficient test, an accurate tool to measure tick sensitivity to the chemical bases of acaricides (Drummond et al. 1973; Scott 1995). The results have immediate practical applicability, directing further research on efficient alternatives to control these ectoparasites (Scott 1995).

The results of bioassays 1 and 2 demonstrated that the semi-engorged *R. sanguineus* females were not sensitive to all the concentrations of *Acmeilla oleracea* extract tested, once the concentrations of 1.6 mg/ml, 3.1 mg/ml and 6.2 mg/ml did not cause death or behavioural alterations to the individuals exposed. However, the individuals exposed to the concentration of 12.5 mg/ml began to have their viability gradually affected, and as of the fifth day, dead individuals were found. At higher concentrations (14.3 mg/ml, 16.6 mg/ml, 20 mg/ml, 25 mg/ml, 33 mg/ml, 40 mg/ml, 50 mg/ml and 100 mg/ml), significant mortality was observed and the individuals showed immediate reactions, such as the progressive decrease of locomotor capacity, coordination loss, prostration in inverted position (upside down), stretching of all legs and paralysis. These data indicate that the reduction in the viability of the semi-engorged *R. sanguineus* females was proportional to the extract concentration increase. Similar results were obtained by Castro et al. (2014) for *Rhipicephalus B. microplus* and by Anholeto et al. (2017) for *Amblyomma cajennense*.

With specific regard to the concentration of 100 mg/ml, 100% mortality was observed as early as 24 h following exposure, demonstrating the high efficacy and acaricidal potential of *Acmeilla oleracea* extracts. Castro et al. (2014) studied *R. B. microplus* ticks and reported that the concentration of 1.6 mg/ml of the hexane extract from *Acmeilla oleracea* caused 93% of larval mortality and the concentrations of 79.7 mg/ml caused the death of 50% of engorged females.

The biological activities of *A. oleracea* have been attributed to secondary metabolites, such as spilanthol, an *N*-alkilamide (Ramsewak 1999). Saraf and Dixit (2002) isolated spilanthol from *Acmeilla olareacea* extract and tested it on *Culex* and *Anopheles* larvae and pupae, finding 100% larval mortality for both species 24 h following exposure to the extract at the concentration of 7.5 ppm. Ramsewak (1999) demonstrated the high insecticidal activity of spilanthol against larvae of *Aedes* mosquitoes.

In the present study, the efficacy of *Acmeilla oleracea* extract was influenced by the exposure time. At the concentration of 20 mg/ml, 5%, 10%, 15%, 30% and 50% mortality was observed 2, 3, 5, 6 and 7 days following treatment, respectively. At the concentration of 25 mg/ml, 15% mortality was observed on the first days following treatment, 35% after 5 days, and 55% after 7 days. The concentration of 33 mg/ml caused 5%, 10%, 15%, 35%, 40% and 55% mortality was observed 1, 2, 3, 5, 6 and 7 days following treatment, respectively. At the concentration of 40 mg/ml, 10%, 20%, 30%, 40%, 65% and 80% mortality was observed 1, 2, 3, 5, 6 and 7 days following exposure, respectively. The concentration of 50 mg/ml of the extract caused 15%, 25%, 55% and 90% mortality 1, 3, 5 and 7 days following treatment, respectively. These data indicate that the highest mortality peaks occurred on the fifth and seventh days, when *Acmeilla oleracea* extract presented maximum efficacy. This information is critical for a better comprehension on the plant action against these ectoparasites, serving as a

basis for the development of new acaricidal products or the application of integrated control, associating this chemical with other methods, aiming to reduce the amount of synthetic chemical acaricides used. Similar data were found by Cruz et al. (2016), who observed gradual increase in mortality for *R. B. microplus* and *Dermacentor nitens* treated with *Acmella oleracea* extract.

No mortality, morphological or behavioural alterations were recorded for control groups 1 and 2 throughout the observation period (7 days).

These results demonstrated the acaricidal effect of the extract obtained from *Acmella oleracea*; however, further studies are needed to clarify the action mechanism of this natural compound against arthropods. It is widely reported in the literature that the synthetic chemical acaricides act on specific targets in the nervous system (neurotoxic) or on the chitin biochemical process (Cruz et al. 2016).

Broglio-Micheletti et al. (2009) developed studies using *R. B. microplus* and demonstrated the higher level of efficacy of the jambu extract in comparison with other plants, such as *Azadirachta indica* (neem) and *Cymbopogon citratus* (lemon grass), once it was able to cause mortality of the female ticks on the first days following treatment and significantly decreased oviposition.

The results of bioassay 2, which analysed the efficacy of the *Acmella oleracea* extract on semi-engorged *R. sanguineus* females were subjected to Probit analysis, with LC_{50} and 95% confidence intervals (superior and inferior limits), g (0.95): $LC_{50} = 24.883$ mg/ml (limits: 22.331–28.003). This LC_{50} of the extract of 24.883 mg/ml is very low and close to the LC_{50} of other renowned synthetic chemical acaricides such as fipronil (de Oliveira et al. 2011), which demonstrates the high efficacy of the extract to control pests, i.e. a small amount of the chemical is able to cause 50% mortality of the exposed individuals.

5. Conclusion

Therefore, the present study demonstrated the sensitivity and susceptibility of semi-engorged *R. sanguineus* female ticks to the extract of *Acmella oleracea*. The potential acaricidal effect started at the concentration of 12.5 mg/ml. Mortality rate increased in higher concentrations of the extract, with LC_{50} of 24.883 mg/ml, reaching 100% for the concentration of 100 mg/ml in 24 h, i.e. the effects were dose-dependent. It is important to emphasize that, although lower concentrations of the *Acmella oleracea* extract were sometimes unable to cause death, they caused adverse effects (damages or losses) such as interference in the process of blood intake by hindering the process of fixation to the host, and/or decrease in growth and/or development due to interference in nutrition.

Considering the global appeal for environmental preservation and the promising results regarding the control of *Rhipicephalus sanguineus* ticks, further studies on the use of *Acmella oleracea* as a safe and sustainable alternative to control these ectoparasites should be stimulated.

Disclosure statement

No potential conflict of interest was reported by the authors.

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