BIOSCIENCE JOURNAL

ACARICIDAL ACTIVITY OF *Furcraea foetida* LEAF EXTRACT AGAINST ENGORGED FEMALE *Rhipicephalus (Boophilus) microplus* TICKS

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How to cite: MARTINS, H.A.S., et al. Acaricidal activity of *Furcraea foetida* leaf extract against engorged female *Rhipicephalus (Boophilus) microplus* ticks. *Bioscience Journal*. 2021, **37**, e37031. https://doi.org/10.14393/BJ-v37n0a2021-48254

Abstract

The Rhipicephalus (Boophilus) microplus tick is a major concern for the livestock market worldwide, as it causes serious economic damage. Plant-derived acaricides are an attractive alternative to control this ectoparasite and limit the development of resistance. Therefore, the aim of this study was to evaluate the acaricidal activity of Furcraea foetida leaf extract against engorged female R. (B.) microplus ticks. Our in vitro bioassays showed that the crude extract of leaves from F. foetida caused hemorrhagic swelling and skin lesions in the ticks, and three days of treatment caused 100% mortality. Dose-response assay indicated that this toxicity effect was dose-dependent. Similar effects were observed when the crude extract from F. foetida leaves was denatured by boiling at 100°C. These results suggest that the toxicity of the leaf extract might be associated with thermostable biomolecules. Together, our results show for the first time that the crude extract of F. foetida leaves has acaricidal activity against engorged female R. (B.) microplus ticks and it acts in a dose-dependent manner.

Keywords: Crude extract. *In vitro* bioassay. Ixodidae. Medicinal plants. Mortality.

1. Introduction

Rhipicephalus (Boophilus) microplus (Canestrini 1887) (Acari, Ixodidae) is considered the main tick species that infests cattle in Brazil. Annually, the economic loss caused by this ectoparasite exceeds three billion dollars (Grisi et al. 2014). The control of *R. (B.) microplus* ticks relies mainly on chemical acaricides, such as coumaphos (organophosphate), cypermethrin, permethrin (both of which are synthetic pyrethroids), and amitraz (amidine) (Narahashi 1971; Li et al. 2003; Chen et al. 2007). As a result of their long-term use, this tick species has developed resistance to all major classes of chemical acaricides, thereby reducing the ability to control infestations (Abbas et al. 2014). Moreover, acaricide residues in food products of animal origin and environment pose a significant risk to human health (Marangi et al. 2012). Such issues have challenged researchers to find alternative products to control *R. (B.) microplus*.

Plant extracts have emerged as an alternative to chemical acaricides, and several studies have confirmed their effects on the *R. (B.) microplus* tick, causing mortality among other effects (Borges et al. 2011; Barbosa et al. 2013; Ghosh et al. 2015; Banumathi et al. 2017). Plant-derived acaricides are environmentally friendly, have low toxicity against mammals, and tick resistance development occurs at a slower pace. This latter property is because they contain various active compounds belonging to different

classes of secondary metabolites with different mechanisms of action (Elango and Rahuman 2011; Barbosa et al. 2013; Ghosh et al. 2015; Rosado-Aguilar et al. 2017).

Furcraea foetida (L.) Haw. (Agavaceae) is an evergreen perennial, subshrub, with succulent leaves, and is widely distributed throughout the Caribbean and South America (Crouch and Smith 2011). *F. foetida* has been used for the treatment of hepatitis, uterine conditions, stomachache, wound healing, and rheumatism (van Andel et al. 2007; Nandagopalan et al. 2011). Furthermore, phytochemical analyses of *F. foetida* leaves extracts indicated the presence of different molecules, such as furcreastatin (a steroidal saponin), steroidal glycosides, tannins, flavonoids, and phenolic compounds, that have been associated with cytotoxic and antioxidant activity (Itabashi et al. 2000; Yokosuka et al. 2009; Mathew et al. 2012). Although different studies indicate the presence of bioactive compounds, there are currently no reports on the acaricidal activity of *F. foetida*. Thus, the aim of this study was to evaluate the acaricidal activity of *F. foetida* leaves against engorged female *R.* (*B.*) *microplus* ticks.

2. Material and Methods

Plant material from *F. foetida* was collected in Unaí (16° 21' 27" S, 46° 54' 22" W, altitude 761 m), Minas Gerais, Brazil, between November 2014 and May 2016. To obtain the crude extract, a pestle was used to grind the basal portions of fresh leaves (200 g) from *F. foetida* adult plants, at room temperature (24.7 ± 5.1°C). The extract was filtered using a piece of gauze, transferred to a 15 mL tube, and immediately used for the *in vitro* bioassays. For the dose-response assay, the filtered crude extract was concentrated by lyophilization and then diluted in distilled water to concentrations of 2.5, 5.0, and 10.0 mg mL^{-1} (w v^{-1}). The crude extract was then boiled in a 100°C water bath for 20 minutes.

Engorged female *R. (B.) microplus* ticks were collected from naturally infected cattle in farms of Unaí city. The owners of the cattle had suspended the use of acaricidal treatment 45-days prior collection date (Gazim et al. 2011). Ticks were prepared and used for *in vitro* bioassays within 24 h of the collection. The ticks were washed with distilled water and dried in soft absorbent paper. We selected ticks that presented motility, maximum engorgement, and no morphological anomalies, such as malformations and/or mutilations. Such selection was carefully performed with a stereo-microscope. The ticks were individually weighed and uniformly distributed by weight into three replicates, each containing five ticks.

A modified version of Sheppard and Hinkle's method (1987) was used to test the acaricidal activity of crude extract obtained from *F. foetida* leaves against engorged female *R. (B.) microplus* ticks. Briefly, 3.5 mL of each treatment solution was separately added to a layer of filter paper placed on a Petri dish (10 cm diameter, 1.5 cm depth), and any excess of the solution was removed. Subsequently, the engorged female *R.* (*B.*) *microplus* ticks were evenly distributed in the Petri dishes containing the treated filter paper, and then incubated for 7–10 days at room temperature (24.7 ± 5.1°C; relative humidity, 60.2 ± 22.7%). The time and temperature used in this study were based on a previous work with *R. (B.) microplus* (Jonsson et al. 2007). Distilled water was used as negative control. A commercial acaricide composed by Cypermethrin was diluted with distilled water to a final concentration of 0.3 mg mL^{-1} and used as a positive control. All experiments were independently repeated at least three times.

The viability of the ticks was carefully checked daily using a stereo-microscope, and the mortality rate was recorded by counting the number of dead ticks in each treatment. The ticks were considered to be dead by the presence of cuticular darkness, hemorrhagic swelling, skin lesions, lack of movement, and no reaction to any external stimuli. Finally, the mortality rate was presented as the total percentage. Exposure to the different treatments resulted in a binary response of either death or survival of the ticks; therefore, the results obtained in the bioassays were analyzed using the Chi-square test. A p-value of < 0.05 indicated statistical significance.

3. Results

We evaluated the effect of *Furcraea foetida* leaf extract on engorged female *Rhipicephalus (Boophilus) microplus* ticks. Morphological observations showed cuticle darkness and hemorrhagic swelling in the ticks exposed to the crude extract for three days, and lesions appeared on the skin surface of ticks after five days of treatment (Figure 1A). In contrast, in non-treated ticks, the cuticle color remained normal

and there were no signs of hemorrhagic swelling or skin lesions (Figure 1B). Moreover, the treated ticks showed no signs of movement and did not react to any external stimuli, whereas the movements and reactions of the non-treated ticks were normal.

The activity of the *F. foetida* crude extract against engorged female *R.* (*B.*) *microplus* ticks was assessed by counting the number of dead ticks each day and was expressed as the percentage of mortality. In comparison to distilled water (negative control), the crude extract significantly ($p < 0.05$) increased the mortality of *R.* (*B.*) *microplus* ticks (Figure 1B). In total, 80% of the ticks died two days after treatment with the extract. In contrast, a commercial acaricide composed by cypermethrin (0.3 mg mL^{-1}) that was used as a positive control showed a cumulative mortality of 20% and 60%, 7 and 10 days post-treatment, respectively (Figure 1B). These results indicate that the crude *F. foetida* leaf extract has to contact acaricidal activity against engorged female *R.* (*B.*) *microplus* ticks.

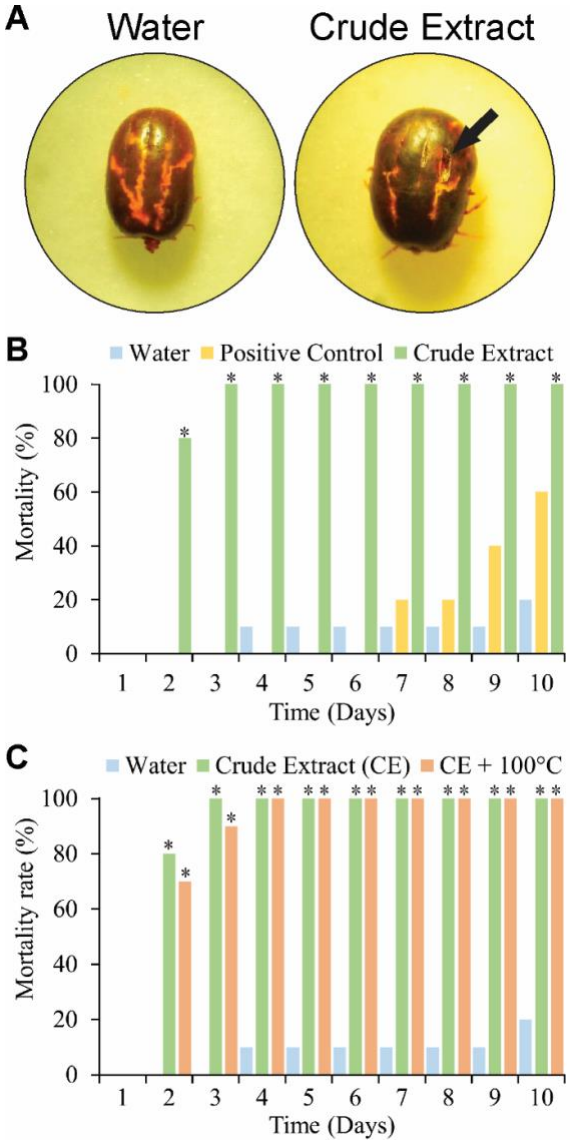

Figure 1. Acaricidal activity of *Furcraea foetida* leaf extract against engorged female *Rhipicephalus (Boophilus) microplus* ticks. A – The crude extract of *F. foetida* leaf causes toxic effects on *R. (B.) microplus* ticks. The ticks were observed by stereo-microscope five days post-treatment with distilled water (negative control) and *F. foetida* leaf crude extract and they showed skin lesions (black arrow). B – The crude extract of *F. foetida* leaf causes the death of *R. (B.) microplus* ticks. The mortality rate of the ticks exposed to water, commercial acaricide (positive control) and crude extract of *F. foetida* leave for ten days. C – The acaricidal activity of *F. foetida* leaf extract is thermo-stably. The mortality rate of *R. (B.) microplus* ticks exposed to water, unheated and heated crude extract of *F. foetida* leaf for ten days. Crude Extract (CE), unheated; CE + 100°C, crude extract heated at 100°C for 20 minutes. Values are expressed as total mortality percentage. Asterisks indicate significance (Chi-square test, $p < 0.05$) compared to the negative control (water).

The activity of the crude extract could be due to the presence of several active biomolecules. To verify whether theses biomolecules are proteins, we treated engorged female *R.* (*B.*) *microplus* ticks with denatured (100°C for 20 min) crude extract of *F. foetida.* Morphological observations showed that the denatured crude extract caused similar toxic effects as the non-denatured crude extract. The denatured extract showed a mortality rate of 70%, which was 10% lower than that observed with non-denatured extract at two days post-treatment but was significantly ($p < 0.05$) higher than that observed with the negative control (Figure 1C). However, the mortality reached 100% at four days post-treatment, indicating that the heating delayed the acaricide effect of the crude extract, but caused no significant change to its activity. This result suggests that the crude extract of *F. foetida* leaves is thermostable, and its acaricide activity is not associated with proteins.

The dose-response assay was carried out using lyophilized preparations of the crude extract, diluted to various concentrations (2.5, 5.0, and 10.0 mg mL^{-1}). The lyophilized crude extract (LCE) caused lesser hemorrhagic swelling and fewer skin lesions on the *R.* (*B.*) *microplus* ticks than the non-lyophilized crude extract. Nevertheless, the results show a gradual and significant ($p < 0.05$) increase in the mortality of ticks, treated with LCE, until four days post-treatment, when the mortality reached 50% (Figure 2). However, the mortality never reached 100%, even at the highest concentration (10.0 mg mL^{-1}) and after 10 days of treatment (Figure 2). These data indicate that the crude extract of *F. foetida* leaves cause the death of ticks in a dose-dependent manner.

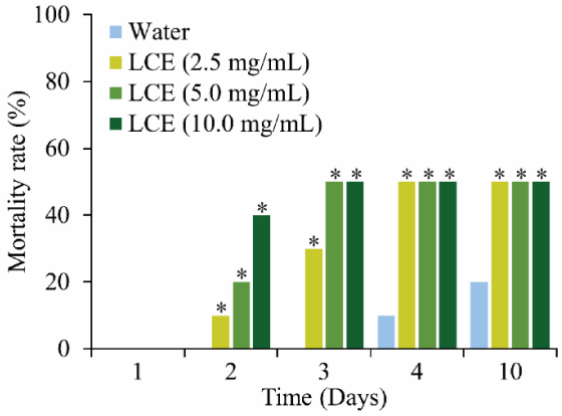

Figure 2. The crude extract of *F. foetida* leaves cause the death of engorged female *Rhipicephalus (Boophilus) microplus* ticks in a dose-dependent manner. The mortality rate of *R. (B.) microplus* ticks exposed to distilled water (negative control) and different concentrations of Lyophilized Crude Extract (LCE) of *F. foetida* leaves. Values are expressed as total mortality percentage. Asterisks indicate significance (Chi-square test, $p < 0.05$) compared to the negative control (water).

4. Discussion

The control of *R. (B.) microplus* ticks has mainly relied on the use of acaricide chemicals, but some of these molecules are very stable and persist in the environment and tissues of treated livestock for long periods, and increase the risk of resistance development (George et al. 2004; Olivares-Pérez et al. 2011). Therefore, alternatives strategies to the control ticks are required, especially that are environment friendly, have fewer negative consequences to the treated animal, and result in lower levels of residue contaminating meat and milk. Plant-derived products are an attractive natural alternative to controlling ticks (George et al. 2014; Rosado-Aguilar et al. 2017). Several plant species have shown *in vitro* and *in vivo* acaricidal activity against *R. (B.) microplus* ticks (Pivoto et al. 2010; Buzatti et al. 2011; Krawczak et al. 2011; Borges et al. 2011; Elango and Rahuman 2011; Barbosa et al. 2013; Adenubi et al. 2016; Banumathi et al. 2017). However, until now, the acaricidal activity of *F. foetida* leaf crude extract against the *R. (B.) microplus* tick has not been tested and reported.

This study evaluated the acaricidal activity of *F. foetida* leaf crude extract against engorged female *R. (B.) microplus* ticks. The extent to which a compound injures or kills the target parasite defines its toxicity level as an acaricide (Roma et al. 2009). Our data show that the crude extract of *F. foetida* leaves has a toxic effect on engorged female *R.* (*B.*) *microplus* ticks, causing hemorrhagic swelling and skin lesions (Figure 1A),

as well as a rapid (two days post-treatment) increase in the rate of mortality (Figure 1B). Similarly, various extracts from other plant species have been shown to exhibit acaricidal activity against *R.* (*B.*) *microplus* ticks within two days (Ribeiro et al. 2007; Ribeiro et al. 2010; Rosado-Aguilar et al. 2010; Fernandez-Salas et al. 2011). Moreover, the mortality rate of engorged female *R.* (*B.*) *microplus* ticks was dose-dependent when treated with different concentrations of the lyophilized crude extract of *F. foetida* leaves (Figure 2). A thorough search throughout the literature (Borges et al. 2011; Adenubi et al. 2016; Banumathi et al. 2017) confirmed that this is the first study to report the acaricidal activity of *F. foetida* leaves against *R. (B.) microplus* ticks. Therefore, this specie could be an option to control tick infestations in livestock, reducing the issues associated with acaricide chemicals.

The contact acaricidal activity of *F. foetida* leaf crude extract (Figure 1 and 2) against *R. (B.) microplus* ticks indicates the presence of bioactive compounds, which acts in a dose-dependent manner. In this work we used cypermethrin at 0.3 mg mL^{-1} as a positive control; this concentration is two times higher than the recommended by the manufacturer of commercial acaricide. Despite that, the cumulative mortality was 20% and 60%, 7 and 10 days post-treatment with cypermethrin (Figure 1B). In parallel, the dose-response assay showed that 2.5 mg mL^{-1} of the lyophilized crude extract caused 50% of mortality after 4 days of treatment (Figure 2). Taken together, these results suggest that there are some potential bioactive compounds in the lyophilized crude extract which are not concentrated and pure such as in positive control but, it still caused toxic effects and mortality on the ticks in less time than the positive control.

Many phytochemical compounds, such as alkaloids, tannins, flavonoids, and proteins have been associated with acaricidal activity in several plant species (Borges et al. 2011; Adenubi et al. 2016; Rosado-Aguilar et al. 2017). In this study, the denatured crude extract of *F. foetida* leaves caused hemorrhagic swelling, skin lesions, and the death of ticks (Figure 1C), suggesting that, in this case, the acaricidal activity might not be due to proteins. In other studies, phytochemical analyses showed the presence of steroidal glycosides, tannins, flavonoids, phenolic compounds, saponins, carbohydrates, and phytosterols in the leaves of *F. foetida*, which were associated with cytotoxic and antioxidant activities (Yokosuka et al. 2009; Mathew et al. 2012). Recently, the acaricidal activity of *Datura metel* fruit extract against *R. (B.) microplus* ticks was attributed to alkaloids and glycosides acting synergistically as acaricides against *R. (B.) microplus* ticks (Ghosh et al. 2015). Similarly to *F. foetida*, *Withania somnifera* possesses antioxidant properties, and the crude extract from its leaves has been shown to have acaricidal activity against synthetic pyrethroid resistant *R. (B.) microplus* ticks (Singh et al. 2014). Other biomolecules, such as the essential oils from *Curcuma longa*, *Zingiber officinale*, *Lippia alba*, *Lippia gracilis,* and *Lippia origanoides,* have also been shown to exhibit activity against *R. (B.) microplus* ticks (Chagas et al. 2016). However, neither alkaloids nor oils were detected in *F. foetida* leaves (Yokosuka et al. 2009; Mathew et al. 2012). Comparing our results with these reports, we hypothesize that the acaricidal activity of *F. foetida* leaves is associated with glycosides and antioxidant compounds. Finally, our *in vitro* bioassays using denatured crude extract suggest that the active compound is thermostable (Figure 1C), an important acaricidal property (Adenubi et al. 2016). Thus, bioassay-guided fractionation studies to identify the bioactive compounds should be conducted to improve the effect of *F. foetida* extracts against *R. (B.) microplus* ticks, and to determine the relevant toxicology and acaricidal mechanisms.

5. Conclusions

In this study, we showed that the crude extract of *F. foetida* leaves has contact acaricidal activity against engorged female *R. (B.) microplus* ticks. The mortality of adult ticks was dose-dependent and was not affected by high temperature. This indicates that *F. foetida* is a promising inexpensive, natural, and environment-friendly phyto-controlling candidate for use against *R. (B.) microplus* ticks. However, complementary experiments to evaluate the acaricidal activity are required, such as the evaluation of other solvents/formulations. In addition, *in vivo* tests are required to verify any toxic effects of the extract on mammals and the presence of trace residues in milk and meat.

Authors' Contributions: MARTINS, H.A.S. and PEREIRA, M.F.: acquisition of data, and analysis and interpretation of data; KONZEN, E.R. and BRONDANI, G.E.: conception and design, and analysis and interpretation of data; CAMPOS, W.F.: conception and design, analysis and interpretation of data, and drafting the article. All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: Not applicable.

Acknowledgments: The authors would like to thank Debora Martins and Renata Luiz Ursine for technical assistance and other people for their critical discussions. The authors also thank the funding for the realization of this study provided by the Brazilian agencies FAPEMIG (Minas Gerais State Research Support Foundation) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico - Brasil).

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Received: 14 April 2019 | **Accepted:** 14 April 2021 | **Published:** 12 June 2021