

In vitro evaluation of the acaricidal activity of propolis against cattle ticks

Avaliação in vitro da ação acaricida da própolis em diferentes concentrações sobre o carrapato bovino

Evaluación in vitro de la acción proparacida del propóleo en diferentes concentraciones en la garrapata bovina

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Abstract

Propolis is a natural substance that is produced by bees and has been used since ancient times for its pharmacological properties. The cattle tick *Rhipicephalus (Boophilus) microplus* responsible for great economic losses by cattle breeding programs. The aim of the present study was to evaluate the *in vitro* effect of propolis extracts on engorged female ticks. The experiment was performed at the Uberaba IFTM. A completely randomized experimental design was used, including eight treatments and five replicates, with a total 40 experimental units, each consisting of 10 engorged females of homogeneous weight. The experimental units were immersed in alcoholic propolis extracts that were diluted with distilled water to the following concentrations: 0, 10, 20, 30, 40, 50, 60, and 70%. A regression analysis was used to analyze the relationship between propolis extract concentration and weight of eggs laid (WEL), hatching percentage (HP), and reproductive efficiency (RE). This demonstrates the viability of propolis as an alternative for the control of cattle ticks, with the 70% extract concentration being most efficient and most effective for controlling *R. microplus* under laboratory conditions.

Keywords: Tick control; *Rhipicephalus (Boophilus) microplus*; Natural substance.

Resumo

A própolis é uma resina natural, produzida por abelhas e vem sendo utilizada desde a antiguidade em virtude de suas várias propriedades farmacológicas. O carrapato *Rhipicephalus (Boophilus) microplus* responsável por grandes perdas econômicas para pecuária bovina. O presente trabalho teve como objetivo avaliar *in vitro* o efeito de diferentes concentrações de extrato de própolis sobre as teleóginas. O experimento foi realizado no IFTM Uberaba. Utilizando o delineamento inteiramente casualizado, com 8 tratamentos e 5 repetições, totalizando 40 unidades experimentais, compostas de 10 teleóginas cada, com pesos homogêneos. Estas unidades experimentais foram submetidas à imersão em solução de água destilada com extrato alcoólico de própolis, nas concentrações de 0%, 10%, 20%, 30%, 40%, 50%, 60% e 70%, as quais foram posteriormente avaliadas quanto a análise de regressão para as variáveis, Peso da Postura (PP), Percentual de Eclodibilidade (PE) e Eficiência Reprodutiva (ER). Isso demonstra a viabilidade da

própolis como uma alternativa para o controle dos carrapatos bovino, na concentração de extrato alcoólico 70% sendo mais eficiente e mais eficaz para controlar *R. microplus* em condições de laboratório.

Palavras-chave: Controle de Carrapatos; *Rhipicephalus (Boophilus) microplus*; Resina natural.

Resumen

El propóleo es una resina natural, producida por las abejas y se ha utilizado desde la antigüedad debido a sus diversas propiedades farmacológicas. La garrapata *Rhipicephalus (Boophilus) microplus* es responsable de grandes pérdidas económicas para la ganadería. El presente trabajo tuvo como objetivo evaluar in vitro el efecto de diferentes concentraciones de extracto de propóleo sobre los teleoginos. El experimento se llevó a cabo en IFTM Uberaba. Utilizando un diseño completamente al azar, con 8 tratamientos y 5 repeticiones, totalizando 40 unidades experimentales, compuestas por 10 teleoginos cada una, con pesos homogéneos. Estas unidades experimentales fueron sometidas a inmersión en solución de agua destilada con extracto alcohólico de propóleo, en concentraciones de 0%, 10%, 20%, 30%, 40%, 50%, 60% y 70%, las cuales fueron posteriormente evaluadas en cuanto a la análisis de regresión para las variables, peso de la postura (PP), porcentaje de incubabilidad (PE) y eficiencia reproductiva (RE). Esto demuestra la viabilidad del propóleo como alternativa para el control de garrapatas bovinas, siendo la concentración de extracto alcohólico 70% más eficiente y más eficaz para el control de *R. microplus* en condiciones de laboratorio.

Palabras clave: Control de garrapatas; *Rhipicephalus (Boophilus) microplus*; Resina natural.

1. Introduction

The word “propolis” is derived from the Greek *pro*, meaning supporting, favoring, and *polis*, meaning city. Propolis, therefore, a substance used for the protection of cities inhabited by the insects, i.e., beehives (Ghisalberti, 1979). The substance is produced by bees and has been used since ancient times for its pharmacological properties. The most well-known propolis is green, but other types of propolis are also found in Brazil, with colors ranging from greenish-yellow to dark brown, owing to the wide variation in their chemical composition (Ghisalberti, 1979). Because propolis is a complex mixture of plant resin, nectar, and pollen, as well as salivary secretions and wax, its qualitative and quantitative chemical composition is quite variable. Propolis includes several phenols and flavonoids, both of which possess pharmaceutical and biological activities, and in addition to its use for human health, the use of propolis in veterinary medicine and zootechnics has also been increasing. In addition to anti-inflammatory properties, propolis also possesses antimicrobial activity, owing to the action of its constituent flavonoids and flavonoid esters. Furthermore, despite the increasing use of propolis in veterinary medicine, few studies have reported its use for the control of ectoparasites of domestic animals, such as cattle ticks (Barreta et al., 2017).

Rhipicephalus (Boophilus) microplus is the main tick species that affects cattle productivity in Brazil, and along with the fly, the species is one of the main vectors of diseases that affect both men and domestic animals. *R. microplus* is also associated with decreases in cattle production, possibly owing to stinging, which causes irritability, and blood loss, which results in weight loss and decreased milk production, in addition to indirect losses from the high costs of chemical control. As a result, there is interest in finding alternatives for the control and elimination of ticks (Mendes et al., 2019).

Tick control is currently performed by applying commercial acaricides. However, the efficacy of these products is decreasing as a result of pesticide resistance (Mendes et al., 2019). Therefore, both prevention programs and the education of clinicians, pharmacists, veterinarians, pesticide companies, and the general public should discuss the impact and consequences of pesticide resistance (Bergeron, Ouellette., 1998).

The chemical control of ticks can also result in human intoxication, as well as the contamination of milk and beef from the treated animals. Therefore, care should be taken to use chemical products correctly. Interest in controlling ticks in domestic animals is traditionally related to anemia, disease transmission, and compromised milk and meat production. These ectoparasites are monoxenous and draw large quantities of blood from their hosts. Because propolis is a phytotherapeutic product, it is permitted for use in organic production systems for the control of specific diseases. Various studies have

investigated the action of propolis for controlling disease in domestic animals, which has subsequently increased interest for the use of natural products in organic animal production (Andreotti, Garcia, Koller., 2019).

Therefore, studies of the use of propolis against ectoparasites of domestic animals, especially cattle ticks, should be considered relevant, since alternatives for the strategic control of these pests are of great importance worldwide. From an ethno pharmacological point of view, propolis is one of the few natural remedies that has been used over a long period of time and by different civilizations (Castaldo & Capasso, 2002). Accordingly, the aim of the present study was to evaluate the *in vitro* effect of alcoholic propolis extracts on *Rhipicephalus (Boophilus) microplus*. The hypothesis being that alcoholic propolis extracts has an efficient tick effect on *Rhipicephalus (Boophilus) microplus*.

2. Methodology

The present study was performed at the campus Uberaba of the Federal Institute of Education, Science and Technology of the Minas Gerais Triangle (Pereira et al., 2018). Propolis collected in the Uberaba region was subject to turbo extraction (Mello et al., 2010), at 50% propolis (w/v) in 96°GL cereal alcohol, filtered until complete removal of propolis.

Engorged females *R. microplus* were manually collected from a herd of naturally parasitized animals on the IFTM Uberaba Campus. Only ≥ 4 mm-long females were collected, and it was confirmed that the host animals had not received any ectoparasitetreatments in the last 25d. The collected specimens were placed in clean plastic containers, identified and transported to the Laboratory of Parasitology of the Federal Institute of Education, Science and Technology of the Minas Gerais Triangle, Uberaba Campus. The specimens were washed under running water using a plastic sieve, dried in paper towels, and separated into 40 sets of 10 females of homogeneous size and weight.

The experiment used a completely randomized design, with eight treatments (0, 10, 20, 30, 40, 50, 60, and 70% diluted extract) and five replicates per treatment, in order to investigate the *in vitro* effects of propolis extract on the tick specimens. Each experimental unit consisted of a Petri dish that contained 10 engorged females. The eight dilutions were prepared in eight separate containers, and ~20-mL aliquots of each dilution were used to immerse theen gorged females corresponding to each experimental unit, resulting in five replicates for each of the eight dilutions, with 10 tick specimens per replicate. After the ticks were immersed for in the appropriate propolis extracts for 5 min, dried using absorbent paper, and fixed in labeled Petri dishes using tape, the total weight of the group(g), and treatment, and placed in a BOD incubator at 27 °C and humidity >80% for 16 d, for laying. For the control treatment (0% extract), the females were immersed in distilled water for the same amount of time as those exposed to the propolis extract treatments.

After 16 d, the laid eggs were removed from the Petri dishes, weighed using an analytical scale, transferred to plastic syringes that were subsequently labeled and sealed with hydrophobic cotton, and kept in a BOD incubator for ~30 d, until larval eclosion. After the 30 d, three homogeneous samples of infertile eggs, larvae, and shells were diluted in 50% glycerin/alcoholin Petri dishes, and larval eclosi on was calculated usinga stereomicroscope. Meanwhile, reproductive efficiency (RE) and product efficacy (PE) were calculated according to Drummond et al. (1973). Therefore, the present study investigated the effects of propolis extracts on the weight of eggs laid (WEL; mg), hatching percentage (HP), RE, and PE.

Because the treatments were quantitative, a regression analysis was performed, and the statistical significance ($p \leq 0.05$) of the regression coefficients was assessed using the t-test. All statistical analyses were performed using R.

3. Results and Discussion

All the propolis extract treatments (10-70%) significantly affected the parameters evaluated (t-test; Table 1).

Table 1. Regression models fitted and t-test values for the regression coefficients.

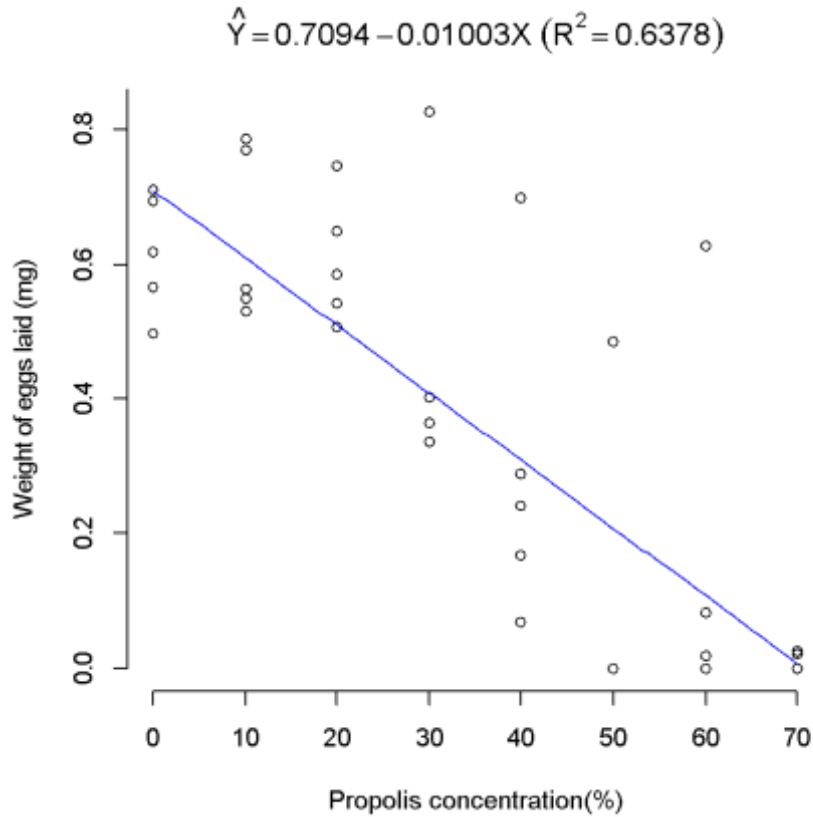
Variable	Parameters	Estimate	Standard		
			error	t	p
WEL	Intercept	0.709385	0.05128	13.834	<2e-16***
	Concentrati n	-0.010028	0.001226	-8.181	6.61e-10**
$R^2 = 0.6378$					
HP	Intercept	87.5402	7.305	11.984	1.78e-14***
	Concentrati n	-1.0829	0.1746	-6.201	3.02e-07***
$R^2 = 0.5030$					
RE	Intercept	405024.6	28759.2	14.083	<2e-16***
	Concentrati n	-5849.6	687.5	-8.509	2.48e-10***
$R^2 = 0.6558$					
PE	Intercept	-16.5375	9.8884	-1.672	0.145471
	Concentrati n	1.6834	0.2364	7.121	0.000386***
$R^2 = 0.8942$					

*WEL = weight of eggs laid; HP = hatching percentage; RE = reproductive efficiency; PE = product efficacy. "***" and "****" indicate significant differences at $p \leq 0.01$ and 0.001 , respectively. Source: Authors.

The model fitted to the relationship between propolis extract concentration and WEL indicated that the WEL under the control treatment (0%) was 0.7094 mg and WEL decreased by 0.01003 mg for each increasing unit of extract concentration (Figure 1). The lowest WEL (0.007425mg) was observed under the 70% extract treatment. This low WEL was also found in a similar study, carried out by Santana et al (2019), in groups of engorged females submitted to the application of the association cypermethrin 15% + chlorpyrifos 25% and citronella 1%, being this association extremely efficient, when compared to other products surveyed, in the control of bovine tick.

This demonstrates that the propolis extracts were efficient in decreasing the WEL of engorged females, and the tested concentration of propolis extracts could, therefore, be expected to decrease the infestation of ticks on cattle and, subsequently, to reduce tick-associated economic losses.

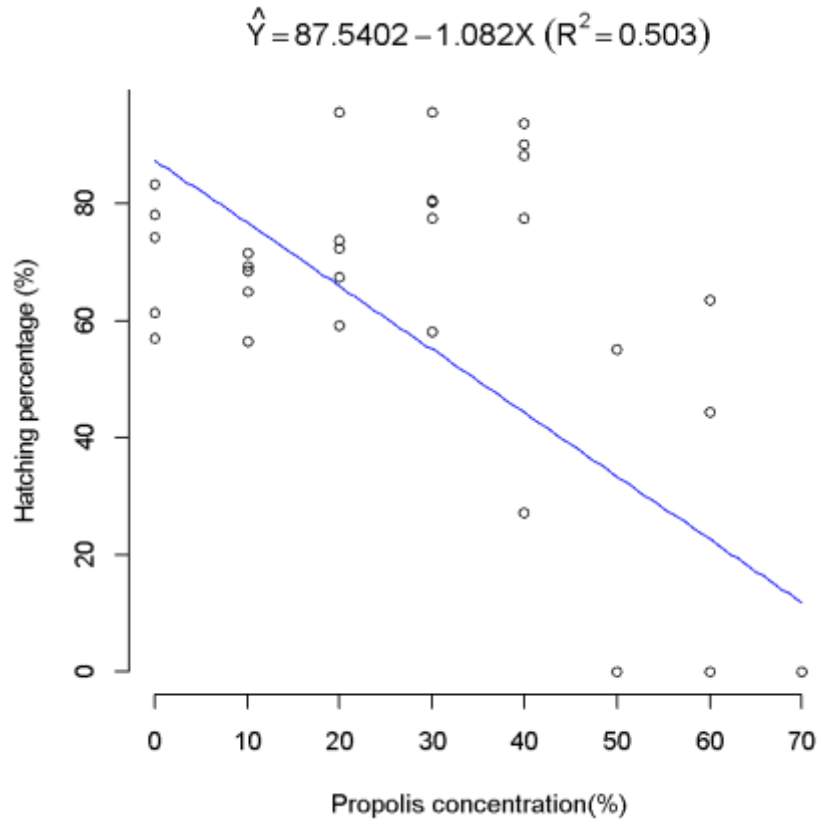
Figure 1. Regression model of the relationship between weight of eggs laid.



(Y-axis) and propolis concentration (X-axis). Fonte: Autores. (2021).

The model fitted to the relationship between propolis extract concentration and HP indicated that HP decreased by 1.0829% for each increasing unit of extract concentration (Figure 2).

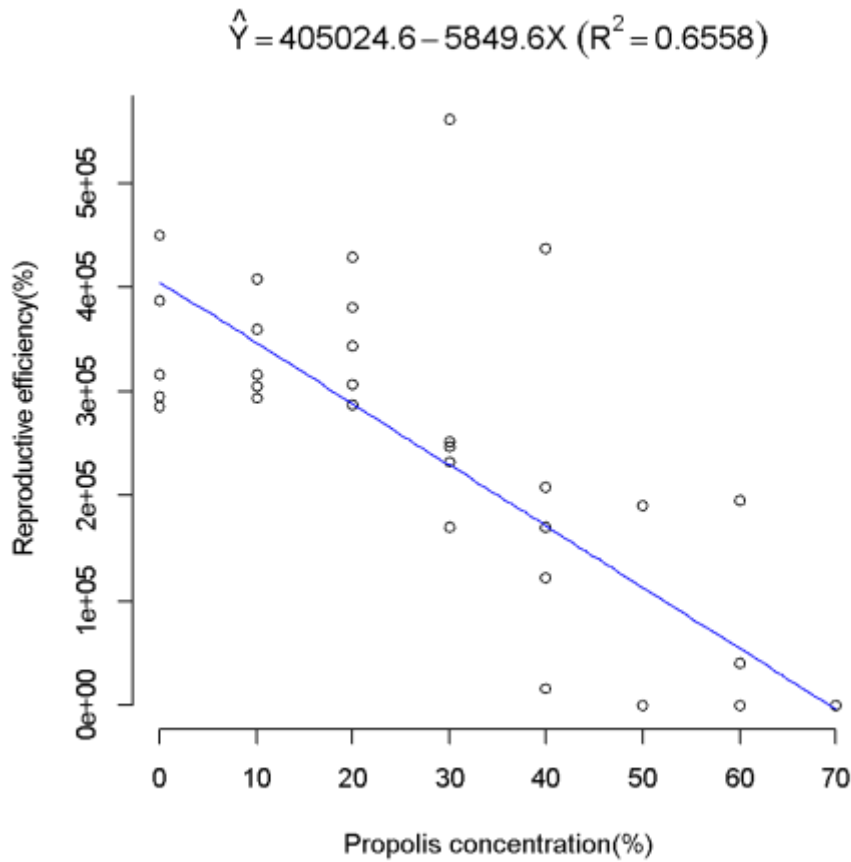
Figure 2. Regression model of the relationship between hatching percentage.



(Y-axis) and propolis concentration (X-axis). Fonte: Autores (2021).

The lowest HP (0.073%) was observed under the 70% extract treatment. Meanwhile, the regression model fitted to the relationship between propolis extract concentration and RE indicated that RE decreased by 5849.6 units for each increasing unit of extract concentration (Figure 3). Together, these results indicate that propolis extracts are an important alternative to chemical acaricides for the control of cattle ticks.

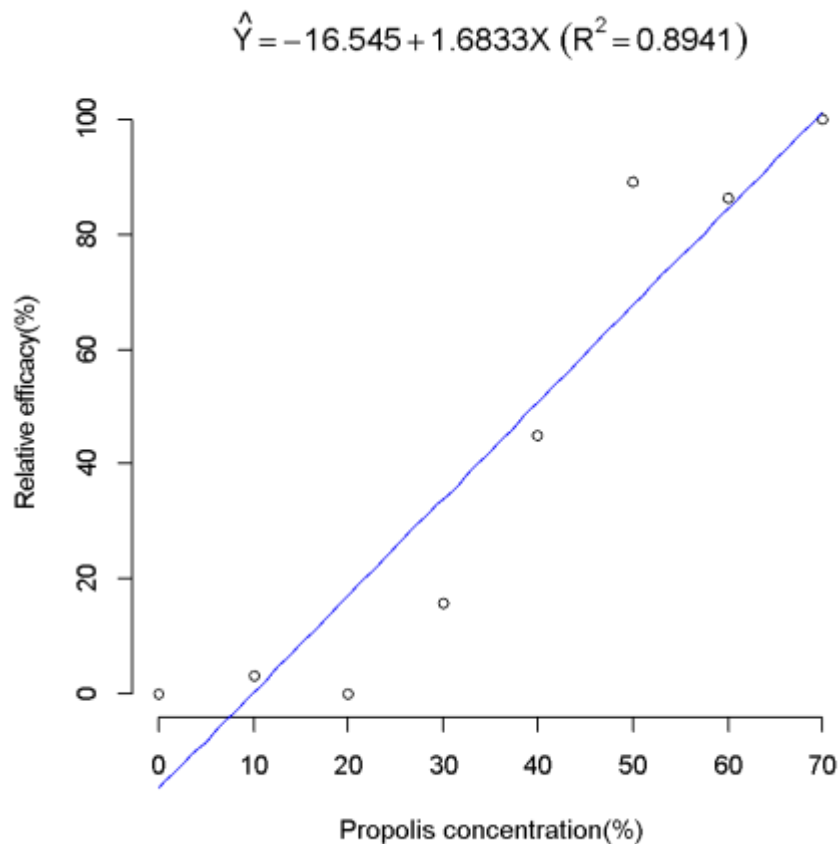
Figure 3. Regression model of the relationship between reproductive efficiency.



(Y-axis) and propolis concentration (X-axis). Fonte: Autores (2021).

The regression model fitted to the relationship between propolis extract concentration and PE indicated that the propolis extract efficacy increased with increasing concentration (Figure 4). The highest PE (100%) was observed under the 70% extract treatment, where as the Ministry of Agriculture (2021), recommends that chemical products should exhibit PE values of 95% to be considered effective. However, there is no specific legislation for evaluating the efficacy of products, such as propolis.

Figure 4. Regression model of the relationship between product efficacy.



(Y-axis) and propolis concentration (X-axis). Fonte: Autores (2021).

In contrast to the present study, Pacheco (2006) reported that propolis had no significant effects on tick mortality or WEL at concentrations 1, 1.5, 2, and 2.5%. However, this difference may have resulted from the low propolis concentrations, compared to those used in the present study (10-70%), or from differences between tick strains.

The concentration-dependent effects of the propolis extracts (lower WEL, HP, and RE; higher PE) could result from propolis' antiparasitic properties (Principal et al., 2002; Loureiro, 2007; Heinzen et al., 2012; Barreta et al., 2017), which are attributed to flavonoids, the major components of propolis (Mazzuco et al., 1996; Castro et al., 2007; Marcucci et al., 1996).

One advantage of using phytotherapeutic products, rather than synthetic acaricides, for the control of cattle ticks is that the activities of natural products are the result of varied and complex chemical compositions, which delays the development of pesticide resistance (Gonçalves, Huerta, Freita, 2016).

We emphasize that the researched literature describes the identification of the acaricidal potential of the crude extract of the genipap fruit (*Genipa americana*) as a control of bovine ticks *Rhipicephalus (Boophilus) microplus* (Bispo, Almeida, Nunes, 2020). Another study, that the essential oil of the leaves of *Psidium rufum* DC has the potential to control *Rhipicephalus microplus* in the life cycle of the larval phase, inferring a promising plant for the application and development of products against bovine ticks (Branco et al., 2020). The essential oil of the leaf and flower of *B. dracunculifolia* also demonstrated stability and application potential for the control of bovine ticks (Cazella et al., 2020). Studies that corroborate our results.

4. Conclusion

The present study examined the effect of alcoholic propolis extracts on WEL, HP, and RE. The 70% propolis extract was the most effective in decreasing all three parameters evaluated and, therefore, was the most effective for controlling *R. microplus* under laboratory conditions. Through study we can infer that 70% propolis has good prospects to be used, and effectively, without control of the bovine tick. We suggest for future studies the evaluation in different regions, and monitoring over time.

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