SIEMBRA
REVISTA DE CIENCIAS
DE LA VIDA

Original Article

Effect of aqueous extract of *Ocotea quixos* leaf on *Rhipicephalus microplus in vitro*

Efecto del extracto acuoso de la hoja de *Ocotea quixos* sobre *Rhipicephalus microplus in vitro*

Joshua Alejandro Bohórquez Vargas[1], Kevin Alexander Mena Quinteros [1], Danilo Reni Vinocunga-Pillajo[1], Isabel Viamonte Garces[1], Yasiel Arteaga Crespo[1]

Siembra 13 (1) (2026): e8162
DOI: 10.29166/siembra.v13i1.8162

Received: 28/04/2025
Revised: 15/07/2025 / 20/08/2025
Acepted: 13/11/2025

Check for updates

[1] Universidad Estatal Amazónica. Facultad de Ciencias de la Vida. 160101. Puyo, Pastaza, Ecuador.

* *Correspondence:* rd.vinocungap@uea.edu.ec

Abstract

Ticks seriously affect cattle in tropical areas, being *Rhipicephalus microplus* (Ixodida: Ixodidae) the most relevant species due to its ability to transmit diseases and generate significant economic losses. The objective of this study was to evaluate the tick-killing action of the aqueous extract of the leaf of *Ocotea quixos* against *R. microplus*. A completely randomized design with a factorial arrangement was employed, in which three concentrations of the hydrolate (25%, 62.5%, and 100%) and three immersion times (1.0, 3.5, and 6.0 minutes) were analyzed, along with positive and negative controls. The tests were conducted under controlled conditions, and mortality was measured every 4 hours for 7 days. The results showed that the hydrolate concentration and its interaction with exposure time significantly influenced tick mortality ($p < 0.0001$), highlighting the 100% treatment applied for 6 minutes, which achieved 87% mortality, a value comparable to the positive control (99.1%). The predictive model showed a high fit ($R^2 = 0.9973$), allowing the effects of the treatment to be accurately anticipated. It was verified that immersion time only improves efficacy at low concentrations. The aqueous extract of *O. quixos* represents an effective natural alternative to synthetic acaricides, contributing to the sustainability of livestock production through biological control strategies with a lower environmental impact.

Keywords: Amazon cinnamon; cinnamaldehyde; hidrosol; ishpingo; tick.

Resumen

Las garrapatas afectan gravemente al ganado bovino en zonas tropicales, siendo *Rhipicephalus microplus* (Ixodida: Ixodidae) de las especies más relevantes por su capacidad de transmitir enfermedades y generar pérdidas económicas significativas. El objetivo del trabajo fue evaluar la acción garrapaticida del extracto acuoso de la hoja de *Ocotea quixos* contra *R. microplus*. Se utilizó un diseño completamente aleatorizado con arreglo factorial, donde se analizaron tres concentraciones del hidrolato (25%, 62,5% y 100%) y tres tiempos de inmersión (1,0; 3,5 y 6,0 minutos), además de controles positivos y negativos. Las pruebas se realizaron en condiciones controladas y la mortalidad fue medida cada 4 horas durante 7 días. Los resultados evidenciaron que la concentración del hidrolato y su interacción con el tiempo de exposición influyeron significativamente en la mortalidad de las garrapatas ($p < 0,0001$), destacando el tratamiento con 100% durante 6 minutos, con un 87 % de mortalidad, valor similar al control positivo (99,1%). El modelo predictivo

SIEMBRA
https://revistadigital.uce.edu.ec/index.php/SIEMBRA
ISSN-e: 2477-8850
Frequency: half-yearly
vol. 13, issue 1, 2026
siembra.fag@uce.edu.ec

mostró un alto ajuste (R^2 = 0,9973), permitiendo anticipar con precisión los efectos del tratamiento. Se comprobó que el tiempo de inmersión solo mejora la eficacia en bajas concentraciones. El extracto acuoso de *O. quixos* representa una alternativa natural eficaz frente a acaricidas sintéticos, aportando a la sostenibilidad de la producción ganadera mediante estrategias de control biológico con menor impacto ambiental.

Palabras clave: canela amazónica; cinamaldehído; garrapata; hidrolato; ishpingo.

1. Introduction

Arthropod ectoparasites that feed on blood, such as ticks, fleas, mites, and mosquitoes, are vectors of pathogens that affect human and animal health worldwide (Mendoza-Roldan et al., 2020). Among these, ticks of the family *Ixodidae* are commonly known as hard ticks due to the shield that covers the entire dorsal surface of the male and only the anterior third in females (de la Cruz Díaz et al., 2023). This family accounts for approximately 78% of all known tick species, including the most economically important ones (Kasaija et al., 2021). Ticks affect 80% of the global cattle population and are associated with numerous health and economic impacts (Sultankulova et al., 2022). Consequently, they cause financial losses of approximately USD 13.9–18.7 billion per year worldwide (Betancur Hurtado & Giraldo-Ríos, 2019).

In Mexico, 65% of cattle are infested with *Rhipicephalus microplus* (Canestrini, 1888) (*Ixodida: Ixodidae*) and are susceptible to tick-borne pathogens, resulting in annual economic losses of 573.6 million dollars (Lagunes-Quintanilla et al., 2024). Meanwhile, in Brazil, it is the most important ectoparasite affecting cattle and has caused estimated losses exceeding three billion dollars (Klafke et al., 2024).

In Ecuador, livestock farming plays a fundamental role in economic and social development, having adapted to a diversity of production systems and to the contrasting conditions that characterize the country's four regions (Maya-Delgado et al., 2020). The main pathogens transmitted by ticks cause the following diseases: bovine babesiosis, bovine anaplasmosis, bovine theileriosis, and hemorrhagic diseases in cattle (Henker et al., 2020; Souza et al., 2013). In the country, more than 75% of cattle are in tick-infested areas (Rodríguez Trujillo et al., 2021), resulting in multimillion-dollar economic losses each year. These losses are mainly due to mortality caused by diseases transmitted by these infected ectoparasites, as well as the consequent reduction in meat and milk production.

Added to this problem is the frequent and indiscriminate use of synthetic biocides, which has favored the proliferation of naturally resistant individuals. This resistance is not induced by the biocides; rather, by eliminating susceptible individuals, only the resistant ones remain, and their proportion increases after each application. In addition, these products have side effects on the environment by contaminating soil and water, affecting local ecosystems, and they may be present in animal-derived foods, as they are excreted in milk or deposited in meat, thereby compromising human health (Paucar-Quishpe et al., 2024).

Authors such as Pérez-Otáñez et al. (2024), identified resistance levels to acaricidal products on 96 cattle farms in the country, 72% of which showed resistance to amitraz, 70% to ivermectin, and 64% to alpha-cypermethrin. Tick resistance to chemical acaricides represents a problem for livestock production, especially in tropical and subtropical regions where species such as *R. microplus* are common (Castañeda Arriola et al., 2021). This resistance compromises the effectiveness of treatments, increases production costs, and poses environmental and human health risks due to the accumulation of chemical residues that are excreted in milk and other bovine tissues (Klafke et al., 2024).

Ocotea quixos (Lam.) Kosterm. (Laurales: Lauraceae) is an aromatic botanical species native to and cultivated in Ecuador, widely distributed in the Andean and Amazonian regions, especially in the Amazon provinces (Gilardoni et al., 2021). Commonly known as "ishpingo" (from the Quechua *ishpinku*), its essential oils have been identified as having various biological activities, including antimicrobial, antioxidant, antiplatelet, anti-inflammatory, and larvicidal properties against *Aedes aegypti* (Valarezo et al., 2021).

The use of plant extracts as acaricides has become a promising alternative to the problems associated with the use of synthetic chemical products (Hagg et al., 2024). Extracts obtained from plants contain bioactive compounds, such as alkaloids, flavonoids, and tannins, which have demonstrated toxic effects on ticks (Quadros et al., 2020).

Hydrolate, also known as floral water, is the water obtained from the condensation of plants during the water distillation or steam distillation process used to extract essential oils (Aćimović et al., 2020). Although hydrolate and essential oils are not miscible, they often share similar properties, containing a reduced proportion of aromatic compounds and other water-soluble chemical elements, which confer appreciable functional properties. Wang et al. (2024), in characterizing the oil and hydrolate of *Cinnamomum osmophloeum* Kanehira, reported that the chemical composition of the hydrolate closely resembles that of the essential oil, identifying trans-cinnamaldehyde (65.03%), *trans-cinnamyl* acetate (7.57%), and coumarin (4.31%) as the main volatile compounds. This indicates the presence of potentially potent compounds within the aqueous fraction that constitutes the hydrolate.

The literature review identified authors such as Kemal et al. (2020), who evaluated methanolic extracts of *Vernonia amygdalina*, *Calpurnia aurea*, *Schinus moll*, and *Ricinus communis* against species of the genus *Rhipicephalus*, showing that concentrations of 50 and 100 mg mL^{-1} achieved significant mortality similar to diazinon after 24 hours. In contrast, Jian et al. (2022) evaluated extracts from six plants against *Dermanyssus gallinae*, with *Syzygium aromaticum* and *Leonurus artemisia* standing out for achieving 100% mortality in contact tests at a concentration of 1 g mL^{-1}.

The use of *O. quixos* has been shown to be an important source of bioactive compounds with potential for the development of natural alternatives in pest management (Scalvenzi et al., 2019). Similarly, studies characterizing Amazonian cinnamon oil conducted by Arteaga-Crespo et al. (2021) highlight the presence of cinnamaldehyde as the main compound with insecticidal effects against termites. Meanwhile, Alvarado Aguilar et al. (2019) emphasize the antimicrobial, antifungal, and antioxidant properties of its essential oil, which contains cinnamaldehyde as the principal active component.

According to the findings reported by Quirós-Monge et al. (2025), this previously mentioned chemical compound has the ability to denature proteins and inhibit the production of enzymes such as acetylcholinesterase, which are crucial for cholinergic nerve transmission in arthropods. It also affects cell membrane permeability, an alteration capable of disrupting physiological processes, compromising homeostasis, and ultimately leading to progressive paralysis and death of ticks.

Therefore, its evaluation as an acaricide makes it possible to develop sustainable natural products, reducing dependence on synthetic chemicals and their environmental impacts. In addition, it makes use of Amazonian resources to strengthen integrated pest management strategies.

Based on the aforementioned premises, the objective of this study was to evaluate the tick-killing activity of the aqueous leaf extract of *Ocotea quixos* against *Rhipicephalus microplus*.

2. Materials and Methods

2.1. Localization

The experimental investigation was done at the laboratories of Chemistry (extraction of the hydrolate of *O. quixos*) and Biology (identification and counting of ticks) of the Universidad Estatal Amazónica [UEA], located in Puyo, Pastaza. Main address: Vía Napo km 2 ½, Paso Lateral S/N, where the experimental procedures and pertinent observations were taken for the analysis (Figure 1).

2.2. Research design

The study is experimental and quantitative because it manipulated the concentration of the hydrolate and the immersion time to analyze its effect on tick mortality. This design established a cause-effect relationship between control variables and obtained responses (Torales & Barrios, 2023). Moreover, it has an exploratory and applied focus, evaluating a natural extract as a sustainable alternative in the management of ectoparasites.

2.3. Recollection of Plant Material

Fresh leaves of *O. quixos* were collected in the properties of UEA. A leave cutter was used to collect the leaves, obtaining a total of 6.5 kg. The collected leaves were placed inside interwoven bags with breathable and clean plastic thread to avoid contamination. They were immediately transported to the laboratory for processing in the FIGMAY brand steam distillation oil extractor, Buenos Aires, Argentina.

2.4. Extraction of the Hydrolate

The hydrolate was obtained by steam distillation, following the methodology described by Briones-Sornoza and Guerrero-Intriago (2019), using an essential oil extractor in the Chemistry Laboratory of the UEA. Two liters of water were added to the extractor, the lids were secured to prevent steam leakage, and fresh *O. quixos* leaves were placed inside the equipment. The applied heat generated steam that ruptured the plant cells, releasing volatile compounds that condensed into liquid form. Two extraction cycles were performed. The first ran from 10h30 to 12h35, and the second from 13h40 to 15h00, yielding a total of 1,387.7 g of hydrolate. The product, labeled "Ocotea Hydrolate," was sealed with parafilm and stored at 5 °C to prevent degradation.

The hydrolate solutions were prepared following the equation [1] and stored inside precipitation glasses of 600 mL. The concentrations were 100% (pure hydrolate, no dilution), 62.5% (312.5 mL of pure hydrolate plus 187.5 mL water) and 25.0% (125 mL of pure hydrolate plus 375 mL water).

$$C_1 \times V_1 = C_2 \times V_2 \quad [1]$$

Where, C_1: Initial concentration of hydrolate (100%), V_1: Volume of pure hydrolate used, C_2: final desired concentration (25% or 62.5%), V_2: total Volume (500 mL).

The hydrolate was not chemically characterized; its composition is inferred based on findings from previous studies that report the presence of potentially potent bioactive compounds from the essential oil within the aqueous fraction that constitutes the hydrolate in different cinnamon species. According to Arteaga-Crespo et al. (2021), this has been described for Amazonian cinnamon (*O. quixos*), and according to Wang et al. (2024), for the hydrolate of *Cinnamomum osmophloeum* Kanehira.

2.5. Recollection, identification and mortality of ticks

Tick collection was carried out at livestock facilities in the province of Pastaza. The ticks were taken from cattle in the groin region, behind the ears, neck, udders, scrotum, and perineal area, in accordance with what was reported by Cuesy León et al. (2021) in infested cattle. A total of 300 ticks were collected, of which 170 were selected and randomly distributed into Petri dishes for the treatments.

Species identification was performed using a EUROMEX NZ1903-P trinocular stereoscope to observe morphological characteristics, with the aid of the taxonomic keys described by Acevedo-Gutiérrez et al. (2020). It was determined that the ticks used in this study corresponded to the species *Rhipicephalus (Boophilus) microplus*, a species very common in the area. The specimens were found at different developmental stages, with predominance of nymphs and adults.

The ticks were randomly distributed into perforated plastic Petri dishes (96 × 16 mm) to allow ventilation, with 10 ticks per dish, totaling 170 ticks. The dishes were labeled according to the corresponding treatments, specifying the dose and exposure time within each hydrolate concentration, according to the proposed design and the treatments indicated in Table 1. The ticks were immersed in the corresponding solutions, with immersion time controlled using a stopwatch. Subsequently, they were removed and returned to their respective Petri dishes.

The evaluation of mortality was done from 08h00, each 4 h, to verify they were dead and not simply immobilized. A fine brush was used to stimulate their

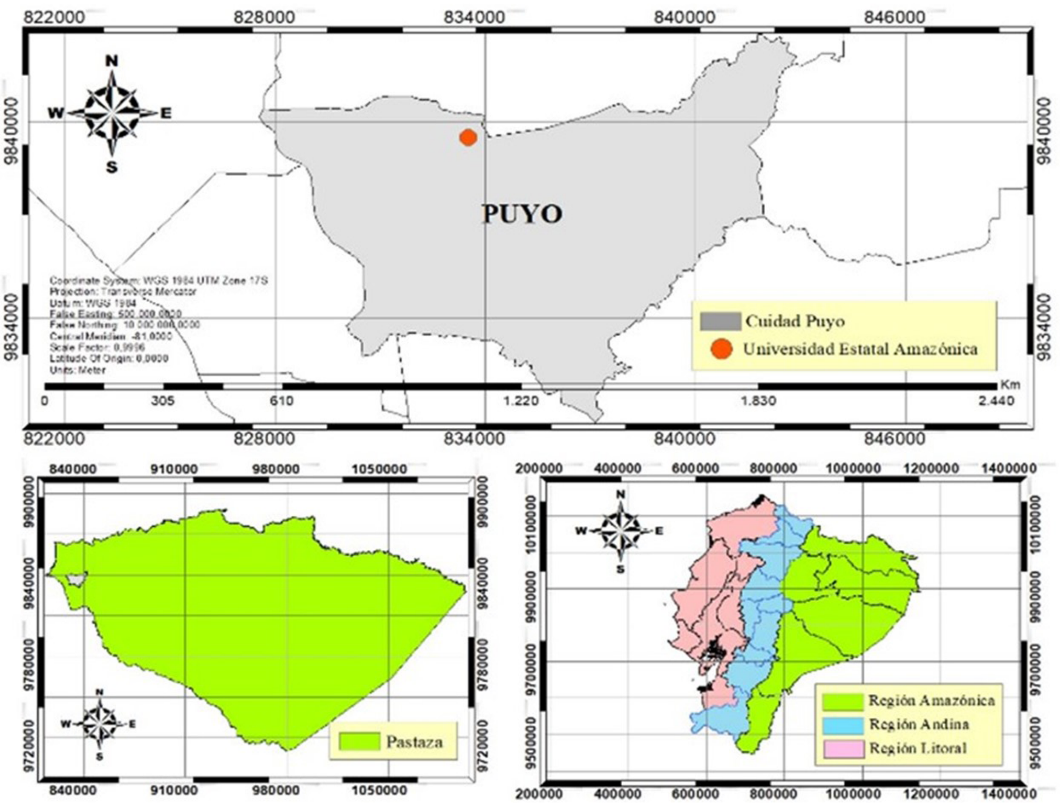

Figure 1. Geographical location of the study area.

mobility through soft mechanical contact. The temperature lab conditions were kept controlled at 28±2 °C, and relative humidity of 80-90% during all the entire lab phase. For the mortality measurements, the equation [2] was used.

$$\%\ dead\ ticks = \frac{Total\ dead\ ticks}{Number\ of\ ticks\ initially\ captured} \times 100 \quad [2]$$

2.6. Experimental Design

The treatments were arranged in a completely randomized experimental design [CRD], following an approach similar to that used by Valenzuela Loor et al. (2024). Three hydrolate concentrations (25.0%, 62.5%, and 100%) were evaluated, with three immersion times (1.0, 3.5, and 6.0 minutes), along with two controls (positive and negative). The controls were subjected to the same immersion time as the treatments, that is, 6 minutes, to ensure homogeneous experimental conditions (Table 1). Distilled water was used as the negative control, and the commercial acaricide Biorboss-Farbiovet was used as the positive control. This product is composed of 95% cypermethrin, dichlorvos (an acetylcholinesterase inhibitor), and fipronil (a GABA-gated chloride channel blocker), and was diluted at 1 mL L^{-1} of water (according to the manufacturer's recommended dose used in livestock production). After the immersion period (6 minutes), the ticks were placed back into Petri dishes, where mortality was recorded for 7 days at 4-hour intervals. Mortality was the only response variable considered to evaluate the effect of the treatments.

2.7. Statistical Analysis

The analysis was performed using Design-Expert® 12 software, where the values of sum of squares, degrees of freedom, mean squares, F-values, and p-values were calculated. Model adequacy was evaluated using the coefficients of determination (R^2 and adjusted R^2), as well as through the calculation of pure error and the Lack of Fit test. Final equations were generated in terms of both coded and actual factors to predict the response according to the levels of each variable.

A factorial Analysis of Variance [ANOVA] was applied considering two factors: the concentration of *O. quixos* hydrolate (Factor A) and immersion time (Factor B), each evaluated at three levels. The design also included the interaction term between factors (A×B), allowing identification of possible combined effects between concentration and time. A significance level of 5% ($p < 0.05$) was used to assess the statistical influence of each term on the response variable.

Table 1. Experimental design with hydrolate concentrations and immersion times.

N°	Hidrolate doses (%)	Immersion Time (min)
1	100	1,0
2	25	1,0
3	100	6,0
4	62,5	3,5
5	62,5	3,5
6	62,5	3,5
7	25	6,0
8	100	1,0
9	100	6,0
10	25	1,0
11	25	6,0
12	25	6,0
13	100	1,0
14	25	1,0
15	100	6,0
Control (Water)	100	6,0
Acaricide	Recommended dose by the manufacturer	6.0

3. Results and Discussions

3.1. Acaricide activity of the hydrolate of *Ocotea quixos*

Factorial ANOVA showed that the concentration of the hydrolate ($p < 0.0001$) and the interaction between concentration and time ($p < 0.0001$) significantly influenced the mortality of *R. microplus*. The concentration factor presented the biggest impact (SS = 39.24), followed by the interaction between the factors (SS = 0.70). Immersion time did not show a significant effect by itself ($p = 0.1182$). The low magnitude of the pure error (SS = 0.1067) allowed for a correct model estimation, whose results explained the observed variability (Table 2). These data showed that effectivity of the hydrolate depends mainly on its concentration and its interaction with the exposure time.

3.2. Tick mortality across different treatments and controls

The results shown that the mortality of *R. microplus* varied in function of the concentration of hydrolate and the immersion time. The treatment with 100% hydrolate for 6 min presented 87.00% mortality, a value close to the positive control (C+) that reached 99.10% (Figure 2). The application of the same concentration for 1 min resulted in 81.00% mortality, showing a high effectiveness even in short times of exposure. These data allow the confirmation of a response from dose-time.

The treatments of 62.5% applied for 3.5 min registered 67.00% mortality, while lower concentrations showed reduced effectiveness. The treatment with 25% of hydrolate for 1 min reached 49.67% mortality, and for 6 min descended to 46.00%. These results show that at lower concentrations, the activity of acaricide decreases even under prolonged immerse times, which reaffirms the direct relationship between dose and observed biological effect.

The negative control (C-) treated with distilled water recorded the least percentage of mortality (0.04%), confirming that the lethal effect in the experiments could be attributed to the hydrolate. These findings coincide with the research done by Miranda Reyes et al. (2023), who used the essential oil from *Cinnamomum zeylanicum* to reach an effectiveness of 100% against the larvae of *R. microplus*. Complementary, in Quilanga and Loja cantons of Ecuador, studies using *Melinis minutiflora* and *Lantana camara* extracts also reported significant mortality in larvae and adults of the same genre (Vacacela-Ajila et al., 2023).

The observed biological activity can be explained by the presence of compounds like (E)-cinnamaldehyde (27.03%) and (E)-cinnamyl acetate (36.44%) (Arteaga-Crespo et al., 2021), known by their insecticidal and fungicidal activities (Alvarado Aguilar et al., 2019). Their toxicity has been associated with the alteration of cellular permeability and the enzymatic inhibition of the arthropod homeostasis. Moreover, Scalvenzi et al. (2019) mentioned that essential oils with 1.8-cineole, sabinene and α-pinene, also present in *O. quixos*, exhibit larvicidal and acaricidal effects against blood-sucking insects.

3.3. Analysis of the factors' effectors

The Half-Normal Plot showed that the concentration of the hydrolate was the factor with higher standardized effect on *R. microplus* mortality with an approximate value of 3.6. The interaction between concentration and time (AB) showed a lesser effect but still significant, while the exposure time (B) was located closer to the limits of the estimated error (Figure 3). These results confirm that concentration is the main factor for the acaricide effectiveness in the treatments, followed by the interaction with immersion time.

According to Arteaga-Crespo et al. (2021), this behavior can be explained by the action of compounds like (E)-cinnamaldehyde and (E)-cinnamyl acetate, which interfere in vital metabolic routes of the ectoparasites and alter the stability of their cellular membranes.

Table 2. Factorial ANOVA for tick mortality.

Source	Sum of Squares (SS)	Degrees of Freedom (DF)	Quadratic Mean (QM)	F Value	P Value	
Model	39,98	3	13,33	999,56	< 0,0001	significant
A-Concentration	39,24	1	39,24	2943,06	< 0,0001	
B-Time	0,0408	1	0,0408	3,06	0,1182	
AB	0,7008	1	0,7008	52,56	< 0,0001	
Pure Error	0,1067	8	0,0133			
Corrected total	40,09	11				

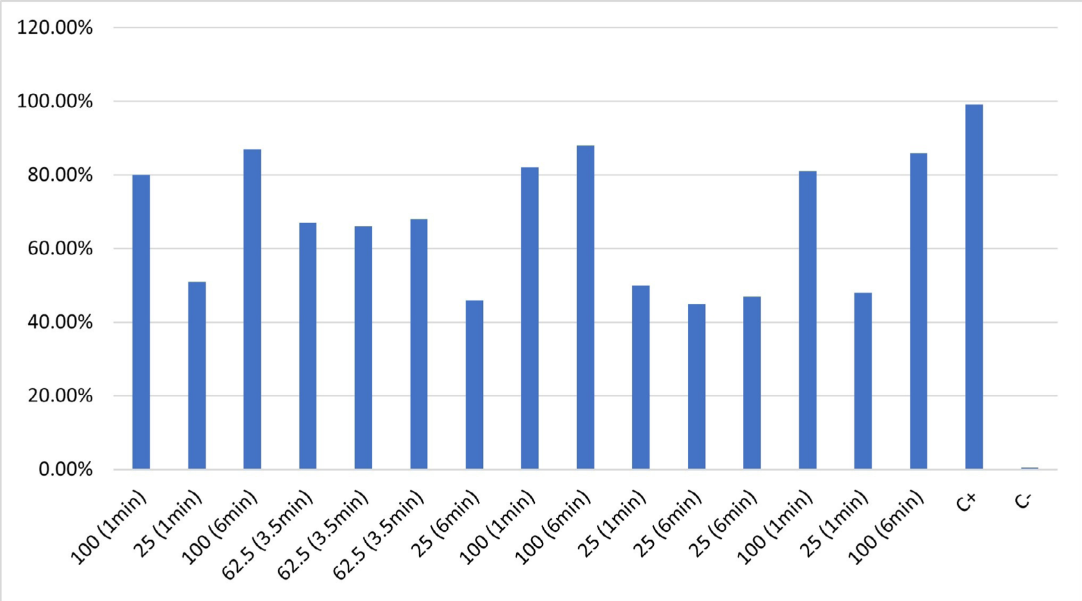

Figure 2. Observed mortality of R. microplus under different concentrations and immersion times of the hydrolate.

3.4. Hierarchy of effects

The Diagram of Pareto shows that the concentration of the hydrolate is the factor with higher impact on the mortality of *R. microplus* with a t value of around 54.25. The interaction between concentration and time (AB) also presented a significant effect, while exposure time (B) did not exceed the limit of Bonferroni, so it is considered statistically insignificant (Figure 4). This hierarchy reaffirms that the effectiveness of the treatment depends mainly on the concentration of the hydrolate, while time acts in combination, but not in isolation. According to Durán Aguirre et al. (2020), the toxicity of the essential oils varies depending on the dose and the interaction of their active components and the physiology of the target arthropod.

3.5. Interaction between factors

The interaction between factors shows that the concentration of the hydrolate and immersion time influence in mortality. The red line (6 min) and black line (1 min) show a progressive increase in mortality as concentration increases, confirming that the most relevant effect corresponds to the quantity of the applied compound (Figure 5). Using high concentrations (100%), it is observed a high mortality independent from time, while at lower concentrations (25%), the exposure time slightly improves the response, although without reaching comparable lethal levels.

These results show that the effect of time only becomes more significant when the dose is reduced due to the necessity of greater absorption to achieve a sufficient toxic effect. In these cases, factors such as individual resistance, oviposition, and biological variability may influence observed mortality. These findings align with those reported by González Puetate et al. (2023), who found that essential oils affect arthropod physiology by interfering with essential metabolic processes and reducing viability. This supports the use of plant extracts as an alternative to synthetic acaricides.

3.6. Distribution of the mortality of the predictive model

In the contour (Figure 6A) and response surface (Figure 6B) plots, it is observed that the highest mortality of *R. microplus* was reached with higher concentrations than the 85% and exposure times close to 6 minutes. The lowest values of both factors were associated with a progressive decrease in the effectiveness of the treatments, reflecting a direct relationship between the increase in concentration and the mortality of the ectoparasite. The blue zone in the graph represents the lowest biological response, while the red zone shows the optimal action point of the hydrolate.

The answer Surface shows a soft, continuous and ascendent distribution. This behavior shows that mortality can be reliably predicted in function of the concentration levels and applied times. These results coincide with the observed ones in experiments with extracts from *Nicotiana tabacum* and *Couroupita guianensis,* where a directly proportional relationship was observed between the concentration and mortality, as well as between exposure time and mortality in larvae of *R. microplus* (Molina et al., 2025).

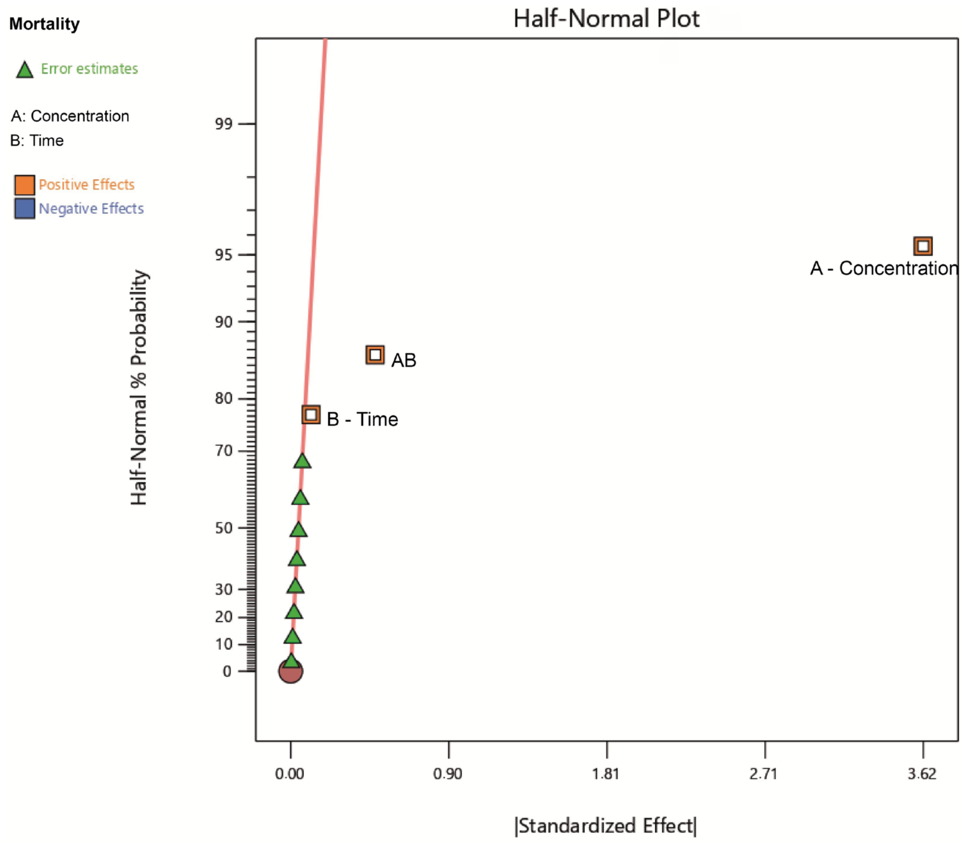

Figure 3. Half-Normal plot of the effects of factors on mortality.

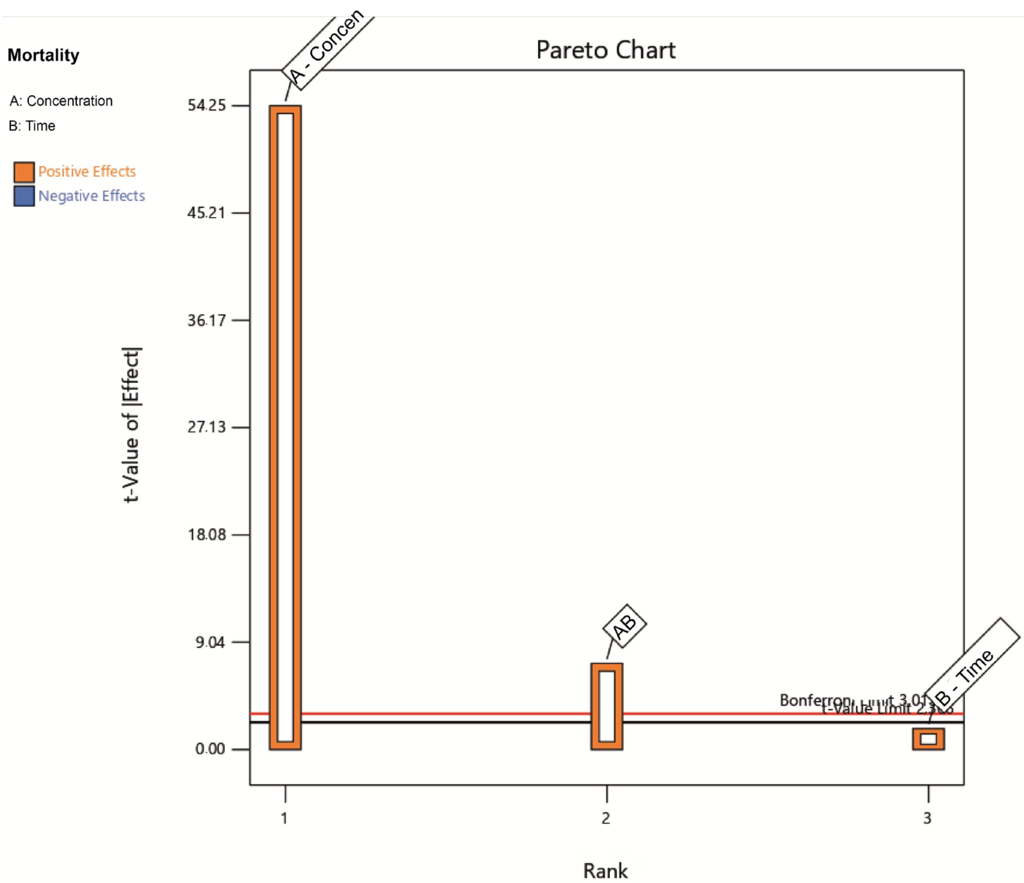

Figure 4. Pareto diagram of the effects of factors on mortality.

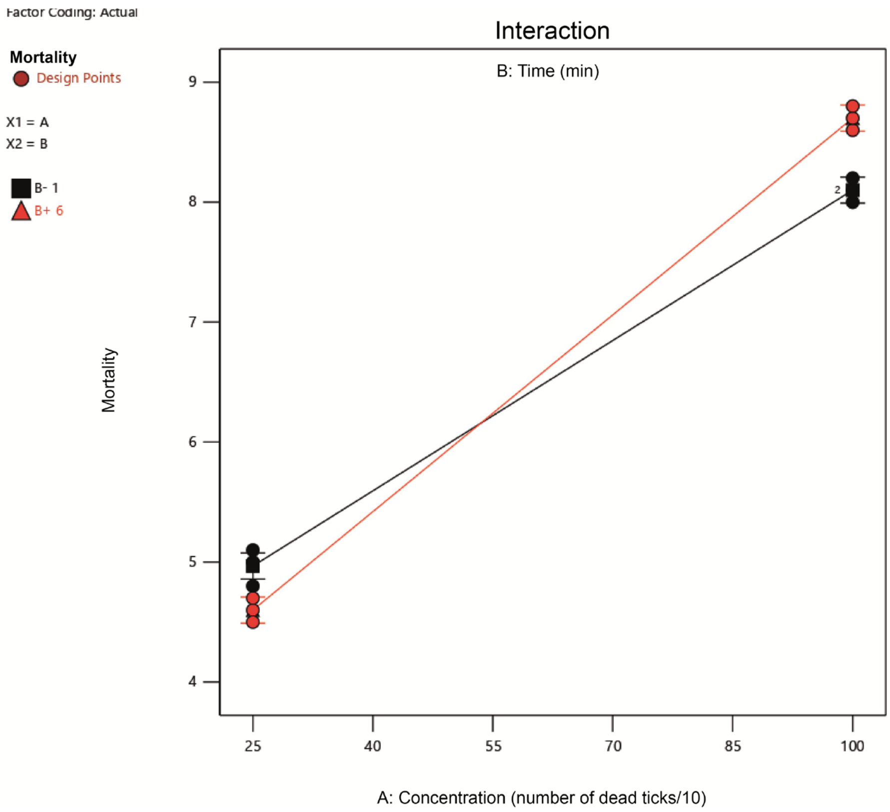

Figure 5. Interaction plot between concentration and time on mortality.

3.7. Comparison between real and predicted values of mortality

The low impact from the *Leverage* values (0.3333) and from Cook Distance (<0.391) show that no observation significantly influenced in the regression fitting model (Table 3). In similar studies, Castillo-Garit et al. (2021) pointed out that the implementation of robust mathematical models allows to minimize the dispersion and improve reliability of the prediction in computational and pharmacokinetics toxicology.

The findings of this study reinforce the applicability of the predictive model to estimate the mortality of *R. microplus* with high precision. This allows for more confident planning of the expected results against variations in concentration of the treatments and exposure time, becoming a useful tool for decision making and strategies of biological control.

The data show that the observations with real values of mortality close to 50% present the highest deviations (order 3), while the treatments with higher values to 80% show tighter predictions. This indicates that the model has higher accuracy in high-concentration conditions and prolonged exposition. These results coincide with reports by Lagunes-Quintanilla et al. (2024), who demonstrated that the effectiveness of the acaricide treatments is influenced by dose and action time on *R. microplus.*

The estimated natural mortality constant (4.06%) in absence of hydrolate is low, what shows an improvement in the adjustment of the predictive model (equation [3]). This value is coherent with other studies, such as the one from De Marchi et al. (2023), where the mortality in the negative control did not exceed 10%. Low interception indicates a lower influence of external covariates such as stress by manipulation or environmental conditions inside the Petri dishes, which have been associated with increments in mortality without acaricide intervention (Ojeda-Chi et al., 2010). The consistency between the model and the experimental data support the reliability of fitness and reinforces their utility as a tool to optimize the use of hydrolate in the biological control of *Rhipicephalus microplus*.

$$Mortality = 4.06 + 0.0392 \times Concentration - 0.1378 \times Time + 0.0026 \times Concentration \times Time \quad [3]$$

The model presented a coefficient of determination (R^2) of 0.9973, showing that the 99.73% of the variability in the mortality can be explained by the evaluated factors. Moreover, the adjusted R^2 (0.9963) and the predicted R^2 (0.9940) demonstrate a high capacity of generalization of the model with a difference less to 0.2. which validates its statistical reliability. The coefficient of determination is considered as optimal for the prediction of biological processes according to the criteria established by Li et al. (2021).

4. Conclusions

The acaricidal effect observed represents an alternative for reducing the use of synthetic compounds in the control of *R. microplus*, one of the main health threats in tropical livestock production. The 87.00% mortality achieved with the 100% concentration at 6 minutes of immersion, a value close to the positive control (99.10%), indicates that the plant extract has high biocidal potential under controlled conditions. This effectiveness suggests that the product could be incorporated into integrated pest management programs, prioritizing ecological practices with lower environmental impact and reduced risk of resistance in ectoparasites.

The predictive model, with a coefficient of determination close to 1, indicates a high capacity to explain the biological behavior of the system, which is necessary for decision-making regarding dosage and treatment application. The minimal difference between the adjusted and predicted values supports the model's stability under new experimental conditions. This consistency allows the model to be used as a reliable tool for designing control strategies based on prior simulations, optimizing resources and reducing the number of field trials.

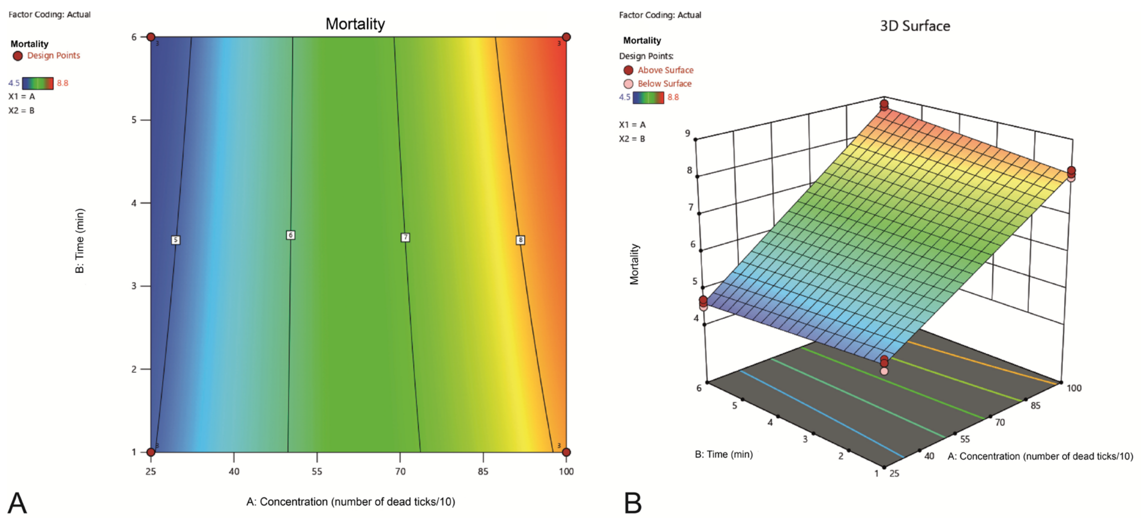

Figure 6. Response surface (A) and contour plot of the effects on the mortality of R. microplus ticks (B).

Table 3. Report of observed and predicted mortality values in the model.

Order	Real Value	Predicted Value	Residue	Leverage	RIE*	REE*	DC*	INF*	Standard Order
1	5,10	4,97	0,1333	0,333	1,414	1,528	0,250	1,080	8
2	5,00	4,97	0,0333	0,333	0,354	0,333	0,016	0,236	11
3	4,80	4,97	-0,1667	0,333	-1,768	-2,118	0,391	-1,498	1
4	4,50	4,60	-0,1000	0,333	-1,061	-1,070	0,141	-0,757	6
5	4,60	4,60	0,0000	0,333	0,000	0,000	0,000	0,000	2
6	4,70	4,60	0,1000	0,333	1,061	1,070	0,141	0,757	7
7	8,00	8,10	-0,1000	0,333	-1,061	-1,070	0,141	-0,757	3
8	8,20	8,10	0,1000	0,333	1,061	1,070	0,141	0,757	5
9	8,10	8,10	0,0000	0,333	0,000	0,000	0,000	0,000	4
10	8,60	8,70	-0,1000	0,333	-1,061	-1,070	0,141	-0,757	9
11	8,70	8,70	0,0000	0,333	0,000	0,000	0,000	0,000	10
12	8,80	8,70	0,1000	0,333	1,061	1,070	0,141	0,757	12

* RIE: Internally Standardized Residual, REE: Externally Standardized Residual, DC: Cook's Distance, INF: Effect on Adjusted Value.

Contributor roles

- Joshua Alejandro Bohórquez Vargas: investigation, methodology, software.
- Kevin Alexander Mena Quinteros: investigation, writing – original draft.
- Danilo Reni Vinocunga-Pillajo: validation, writing – review & editing.
- María Isabel Viamonte Garces: conceptualization, methodology, project administration.
- Yasiel Arteaga Crespo: writing – review & editing.

Data availability

Data will be made available on request

Use of Artificial Intelligence

The authors declare that no artificial intelligence has been used in the preparation of the manuscript.

Ethical Implications

The authors declare that there are no ethical implications.

Conflict of Interest

The authors declare that they have no affiliation with any organization with a direct or indirect financial interest that could have appeared to influence the work reported

References

Acevedo-Gutiérrez, L. Y., Paternina, L. E., Pérez-Pérez, J. C., Londoño, A. F., López, G., & Rodas, J. D. (2020). Garrapatas duras (Acari: Ixodidae) de Colombia, una revisión a su conocimiento en el país. *Acta Biológica Colombiana*, *25*(1), 126–139. https://doi.org/10.15446/abc.v25n1.75252

Aćimović, M. G., Tešević, V. V., Smiljanić, K. T., Cvetković, M. T., Stanković, J. M., Kiprovski, B. M., & Sikora, V. S. (2020). Hydrolates: By-products of essential oil distillation: Chemical composition, biological activity and potential uses. *Advanced Technologies*, *9*(2), 54–70. https://doi.org/10.5937/savteh2002054A

Alvarado Aguilar, M. C., Recalde Coronel, P. C., Leal Alvarado, D. A., Villa Sanchez, F. E., & Tamayo Alcivar, R. (2019). Oil-in-water (O/W) emulsionable concentrate of ishpink (*Ocotea quixos*) with thermodynamic stability. *Revista Caatinga*, *32*(3), 590-598. https://doi.org/10.1590/1983-21252019v32n303rc

Arteaga-Crespo, Y., Ureta-Leones, D., García-Quintana, Y., Montalván, M., Gilardoni, G., & Malagón, O. (2021). Preliminary predictive model of termiticidal and repellent activities of essential oil extracted from *Ocotea quixos* leaves against *Nasutitermes corniger* (isoptera: termitidae) Using One-Factor Response Surface Methodology Design. *Agronomy*, *11*(6),1249 . https://doi.org/10.3390/agronomy11061249

Betancur Hurtado, O. J., & Giraldo-Ríos, C. (2018). Economic and health impact of the ticks in production animals. In M. Abubakar, & P. K. Perera (eds.), *Ticks and Tick-Borne Pathogens*. IntechOpen. https://doi.org/10.5772/intechopen.81167

Briones-Sornoza, H. R., & Guerrero-Intriago, D. A. (2019). Extracción de aceites esenciales de mandarina (*citrus reticulata*) y palo santo (*Bursera Graveolens*) por el método de arrastre de vapor. *Revista Científica INGENIAR: Ingeniería, Tecnología e Investigación*, *2*(3), 14–23. https://doi.org/10.46296/ig.v2i3.0007

Castañeda Arriola, R. O., Álvarez Martínez, J. A., Rojas Martínez, C., Lira Amaya, J. J., Ríos Utrera, Á., & Martínez Ibáñez, F. (2021). Nivel de infestación de *Rhipicephalus microplus* y su asociación con factores climatológicos y la ganancia de peso en bovinos *Bos taurus* x *Bos indicus*. *Revista Mexicana de Ciencias Pecuarias*, *12*(1), 273–285. https://doi.org/10.22319/rmcp.v12i1.5392

Castillo-Garit, J. A., González-Díaz, H., Cañizares-Carmenate, Y., Torrens, F., Pham-The, H., Martínez-López, Y., & Diéguez-Santana, K. (2021). Aplicaciones y potencialidades de los métodos de diseño computacional en estudios ambientales y farmacocinéticos. *Anales de la Academia de Ciencias de Cuba*, *11*(1). http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S2304-01062021000100013&lng=es&tlng=es

Cuesy León, M., Molina Garza, Z. J., Mercado Hernández, R., & Galaviz Silva, L. (2021). Distribución corporal de garrapatas (Acari: Ixodidae y Argasidae) asociadas a *Odocoielus virginianus* (Artiodactyla: Cervidae) y *Ovis canadensis* (Artiodactyla: Bovidae) en tres estados del norte de México. *Revista Mexicana de Ciencias Pecuarias*, *12*(1), 177–193. https://doi.org/10.22319/rmcp.v12i1.528

de la Cruz Díaz, A., González Garduño, R., Vila Pena, M., Castañeda Arriola, R. O., & Maldonado Simán, E. (2023). Prevalencia y diagnóstico de resistencia a ixodicidas en garrapatas de ganado bovino en municipios de Chiapas y Tabasco, México. *Revista Chapingo Serie Agricultura Tropical*, *3*(2), 1–14. https://doi.org/10.5154/r.rchsagt.2023.03.09

De Marchi, B. R., Hennessey, M., Turechek, W., & Smith, H. (2023). A maximum concentration bioassay to assess insecticide efficacy against hemipteran pests of tomato. *Florida Entomologist*, *106*(2). https://doi.org/10.1653/024.106.0211

Durán Aguirre, C. E., Pratissoli, D., Carvalho, J. R. de, Pacheco Damascena, A., Araujo Junior, L. M. de, & Bolsoni Zago, H. (2020). Actividad insecticida de aceites esenciales sobre *Helicoverpa armígera* (Hübner) (Lepidoptera: Noctuidae). *Idesia (Arica)*, *38*(4), 59–64. https://doi.org/10.4067/S0718-34292020000400059

Gilardoni, G., Montalván, M., Vélez, M., & Malagón, O. (2021). Chemical and enantioselective analysis of the essential oils from different morphological structures of *Ocotea quixos* (Lam.) Kosterm. *Plants*, *10*(10), 2171. https://doi.org/10.3390/plants10102171

González Puetate, I., Arévalo Bozada, M. M., Vélez León, M. F., & Acosta Prócel, J. M. (2023). Aceites esenciales, alternativa frente a plagas y enfermedades en apicultura. *LATAM Revista Latinoamericana de Ciencias Sociales y Humanidades*, *4*(5), 30-44. https://doi.org/10.56712/latam.v4i5.1300

Hagg, F., Erasmus, L., & Stoltsz, W. (2024). The potential effect of Garlium GEM HC™ as a tick control agent in cattle. *Journal of the South African Veterinary Association*, *95*(1), 1–6. https://doi.org/10.36303/JSAVA.560

Henker, L. C., Lorenzett, M. P., Fagundes-Moreira, R., Dalto, A. G. C., Sonne, L., Driemeier, D., Soares, J. F., & Pavarini, S. P. (2020). Bovine abortion, stillbirth and neonatal death associated with *Babesia bovis* and *Anaplasma* sp. infections in southern Brazil. *Ticks Tick Borne Dis*, *11*(4), 101443. https://doi.org/10.1016/j.ttbdis.2020.101443

Jian, Y., Yuan, H., Li, D., Guo, Q., Li, X., Zhang, S., Ning, C., Zhang, L., & Jian, F. (2022). Evaluation of the *in vitro* acaricidal activity of Chinese herbal compounds on the poultry red mite (*Dermanyssus gallinae*). *Frontiers in Veterinary Science*, *9*(1),1-11. https://doi.org/10.3389/fvets.2022.996422

Kasaija, P. D., Estrada-Peña, A., Contreras, M., Kirunda, H., & de la Fuente, J. (2021). Cattle ticks and tick-borne diseases: a review of Uganda's situation. *Ticks and Tick-borne Diseases*, *12*(5), 101756. https://doi.org/10.1016/j.ttbdis.2021.101756

Kemal, J., Zerihun, T., Alemu, S., Sali, K., Nasir, M., Abraha, A., & Feyera, T. (2020). *In Vitro* acaricidal activity of selected medicinal plants traditionally used against ticks in eastern Ethiopia. *Journal of Parasitology Research*, *2020*, 10. https://doi.org/10.1155/2020/7834026

Klafke, G. M., Golo, P. S., Monteiro, C. M. O., Costa-Júnior, L. M., & Reck, J. (2024). Brazil's battle against *Rhipicephalus* (Boophilus) microplus ticks: current strategies and future directions. *Revista Brasileira de Parasitologia Veterinária*, *33*(2), e001423. https://doi.org/10.1590/s1984-29612024026

Lagunes-Quintanilla, R., Gómez-Romero, N., Mendoza-Martínez, N., Castro-Saines, E., Galván-Arellano, D., & Basurto-Alcantara, F. J. (2024). Perspectives on using integrated tick management to control *Rhipicephalus microplus* in a tropical region of Mexico. *Frontiers in Veterinary Science*, *11*, 1497840. https://doi.org/10.3389/fvets.2024.1497840

Li, G., Zrimec, J., Ji, B., Geng, J., Larsbrink, J., Zelezniak, A., Nielsen, J., & Engqvist, M. K. (2021). Performance of regression models as a function of experiment noise. *Bioinformatics and Biology Insights*, *15*(1), 1-15. https://doi.org/10.1177/11779322211020315

Maya-Delgado, A., Madder, M., Benítez-Ortíz, W., Saegerman, C., Berkvens, D., & Ron-Garrido, L. (2020). Molecular screening of cattle ticks, tick-borne pathogens and amitraz resistance in ticks of Santo Domingo de los Tsáchilas province in Ecuador. *Ticks and Tick-borne Diseases*, *11*(5), 101492. https://doi.org/10.1016/j.ttbdis.2020.101492

Mendoza-Roldan, J. A., Mendoza-Roldan, M. A., & Otranto, D. (2021). Reptile vector-borne diseases of zoonotic concern. *International Journal for Parasitology: Parasites and Wildlife*, *15*(1), 132-142. https://doi.org/10.1016/j.ijppaw.2021.04.007

Miranda Reyes, P. I., Martínez Ibañez, F., Lagunes-Quintanilla, R. E., & Barrera Molina, A. I. (2023). Efecto ixodicida de los extractos vegetales de *Cinnamomum zeylanicum* y *Tagetes erecta* sobre garrapatas *Rhipicephalus microplus*. *Revista Mexicana de Ciencias Pecuarias*, *14*(4), 905-914. https://doi.org/10.22319/rmcp.v14i4.6394

Molina, W. D., Diaz-Rivas, I. H., y Serrato-Hurtado, C. (2025). Evaluación de susceptibilidad de las garrapatas *Rhipicephalus microplus* a los extractos de *Nicotiana tabacum* y *Couroupita guianensis* en condiciones de laboratorio en Florencia-Caquetá, Colombia. *Revista Científica del Amazonas*, *8*(15), 23-38. https://doi.org/10.34069/RA/2025.15.02

Ojeda-Chi, M. M., Rodriguez-Vivas, R. I., Galindo-Velasco, E., y Lezama-Gutiérrrez, R. (2010). Laboratory and field evaluation of Metarhizium anisopliae (Deuteromycotina: Hyphomycetes) for the control of *Rhipicephalus microplus* (Acari: Ixodidae) in the Mexican tropics. *Veterinary Parasitology*, *170*(3), 348-354. https://doi.org/10.1016/j.vetpar.2010.02.022

Paucar-Quishpe, V., Cepeda-Bastidas, D., Rodríguez-Hidalgo, R., Pérez-Otáñez, X., Perez, C., Enríquez, S., Guzman, E., Ulcuango, F., Grijalva, J., Vanwambeke, S. O., Ron-Garrido, L., y Saegerman, C. (2024). Evaluating the human risks of consumption of foods of bovine origin with ivermectin residues in Ecuador. *Foods*, *13*(21), 3470. https://doi.org/10.3390/foods13213470

Pérez-Otáñez, X., Vanwambeke, S. O., Orozco-Alvarez, G., Arciniegas-Ortega, S., Ron-Garrido, L., y Rodríguez-Hidalgo, R. (2024). Widespread acaricide resistance and multi-resistance in *Rhipicephalus microplus* in Ecuador and associated environmental and management risk factors. *Ticks and Tick-borne Diseases*, *15*(1), 102274. https://doi.org/10.1016/j.ttbdis.2023.102274

Quadros, D. G., Johnson, T. L., Whitney, T. R., Oliver, J. D., y Oliva Chávez, A. S. (2020). Plant-derived natural compounds for tick pest control in livestock and wildlife: pragmatism or utopia? *Insects*, *11*(8), 1-14. https://doi.org/10.3390/insects11080490

Quirós-Monge, M., León-González, I., y Murillo-Rojas, P. (2025). Efecto repelente de aceites esenciales sobre el ácaro *Tetranychus urticae* Koch (Acari: Tetranychidae). *Agronomía Mesoamericana*, *36*, Artículo qwj4k754. https://doi.org/10.15517/qwj4k754

Rodríguez Trujillo, N., Salazar Loor, J. G., Enríquez, S., y Navarro, J.-C. (2021). *Modelo de nicho ecológico de la garrapata Rhipicephalus (Boophilus) microplus incluyendo variables socioambientales dentro de Ecuador continental.* Universidad Internacional SEK. https://repositorio.uisek.edu.ec/handle/123456789/4502

Scalvenzi, L., Radice, M., Toma, L., Severini, F., Boccolini, D., Bella, A., Guerrini, A., Tacchini, M., Sacchetti, G., Chiurato, M., Romi, R., y Di Luca, M. (2019). Larvicidal activity of *Ocimum campechianum*, *Ocotea quixos* and *Piper aduncum* essential oils against *Aedes aegypti*. *Parasite*, *26*, 23. https://doi.org/10.1051/parasite/2019024

Souza, F. de A. L., Braga, J. F. V., Pires, L. V., Carvalho, C. J. S. d., Costa, É. A., Ribeiro, M. F. B., Santos, R. L., y Silva, S. M. M. S. (2013). Babesiosis and anaplasmosis in dairy cattle in northeastern Brazil. *Pesquisa Veterinária Brasileira*, *33*(1), 1-16. https://doi.org/10.1590/S0100-736X2013000900002

Sultankulova, K. T., Shynybekova, G. O., Issabek, A. U., Mukhami, N. N., Melisbek, A. M., Chervyakova, O. V., Kozhabergenov, N. S., Barmak, S. M., Bopi, A. K., Omarova, Z. D., Alibekova, D. A., Argimbayeva, T. U., Namet, A. M., Zuban, I. A., y Orynbayev, M. B. (2022). The prevalence of pathogens among ticks collected from livestock in Kazakhstan. *Pathogens*, *11*(10), 1-16. https://doi.org/10.3390/pathogens11101206

Torales, J., y Barrios, I. (2023). Diseño de investigaciones: algoritmo de clasificación y características esenciales. *Medicina Clínica y Social*, *7*(3), 210-235. https://doi.org/10.52379/mcs.v7i3.349

Vacacela-Ajila, W., Guzmán-Ordóñez, L., Rey-Valeirón, C., Delgado-Fernández, E., Benítez-Gonzales, E., Chamba-Ochoa, H., Ortega Rojas, R., y Ramírez Robles, J. (2023). Composición química y revisión de las propiedades acaricidas de los aceites esenciales de *Melinis minutiflora* y *Lantana cámara*. *Boletín latinoamericano y del Caribe de plantas*, *22*(4), 488-499. https://doi.org/10.37360/blacpma.23.22.4.36

Valarezo, E., Vullien, A., y Conde-Rojas, D. (2021). Variability of the Chemical Composition of the Essential Oil from the Amazonian Ishpingo Species (*Ocotea quixos*). *Molecules*, *26*(13), 3961. https://doi.org/10.3390/molecules26133961

Valenzuela Loor, A. J., Laaz López, D. A., y García Paredes, R. I. (2024). Eficiencia del recubrimiento comestible de almidón de yuca y aceite esencial de canela para la conservación de piña IV gama. *Revista Ciencia y Tecnología El Higo*, *14*(2), 162–177. https://doi.org/10.5377/elhigo.v14i2.19641

Wang, C.-H., Taso, N.-W., Chen, C.-J., Chang, H.-Y., y Wang, S.-Y. (2024). Composition characterization of *cinnamomum osmophloeum* kanehira hydrosol and its enhanced effects on erectile function. *Plants*, *13*(11), 1518. https://doi.org/10.3390/plants13111518