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Susceptibility of *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae) to calcium polysulfide application

Susceptibilidad de *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae) a la aplicación de polisulfuro de calcio

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ARTICLE DATA

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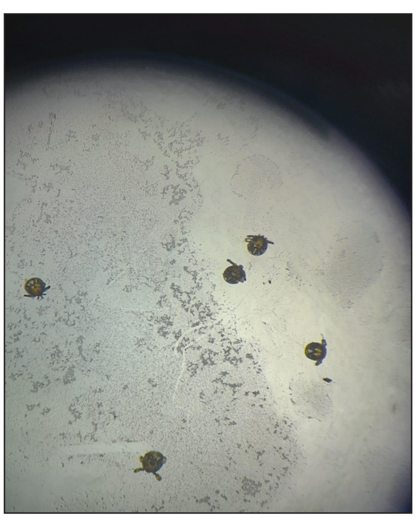

ABSTRACT

Hard ticks are a problem of great importance in the national livestock industry due to their capacity to be vectors of diseases that affect the productivity of animals and humans, becoming an arthropod of veterinary-medical importance. The methods used for their control have not been efficient; therefore, different integrated control alternatives have become crucial to mitigate their population growth. Products such as calcium polysulfide have shown to be an effective alternative in controlling mite populations. The present study was carried out at the Center for Research and Breeding of Natural Enemies of the University of Caldas using nymphs of *Amblyomma cajennense*, arranged in Petri dishes and sprayed with an average volume of calcium polysulfide of 1.3ml. From there, the lethal concentration of calcium polysulfide at 31.5°B, on the individuals of this species was determined. The evaluations were carried out at 24, 48 and 96h after application, finding, by Probit analysis, Cl_{50} of 1.05% and Cl_{95} of 1.35% concentration of the product, which indicates that this compound has the potential to be used in the integrated management plan of *A. cajennense*.

Keywords: Ectoparasites; lethal effect; alternative methods; residuality; ixodicide.

RESUMEN

Las garrapatas duras se presentan como un problema de gran importancia en la ganadería nacional debido a su capacidad de ser vectores de enfermedades que afectan la productividad de los animales y al ser humano, convirtiéndose en un artrópodo de importancia médico-veterinaria. Los métodos que se utilizan para su control no han sido eficientes; por tal motivo, diferentes alternativas de control integrado cobran importancia para mitigar su crecimiento poblacional. Productos como el polisulfuro de calcio han demostrado ser una alternativa eficaz en el control de poblaciones de ácaros. El presente estudio se llevó a cabo en el Centro de Investigación y Cría de

Enemigos Naturales de la Universidad de Caldas usando ninfas de *Amblyomma cajennense*, dispuestas en cajas Petri y asperjadas con un volumen promedio de polisulfuro de calcio de 1,3ml. A partir de allí, se determinó la concentración letal del polisulfuro de calcio a 31,5°B sobre los individuos de esta especie. Las evaluaciones fueron realizadas a las 24, 48 y 96h posteriores a la aplicación, encontrándose, mediante el análisis Probit, la Cl_{50} de 1,05% y la Cl_{95} de 1,35% de concentración del producto, lo que indica que este compuesto posee potencial para ser utilizado en el plan de manejo integrado de *A. cajennense.*

Palabras clave: ectoparásitos; efecto letal; métodos alternativos; residualidad; ixodicida.

INTRODUCTION

According to the Comité Nama Bovina (2021), cattle farming in Colombia occupies 80% of the agricultural land and 28% of the country's water demand. It participates in the sixth line of the GDP of the Colombian economy, being one of the main food sources of the population. Colombia ranks 11th in meat production and has the fifth-largest herd for meat production in America after Brazil, the USA, Argentina, and Mexico; additionally, it is positioned as the 12th nation in milk production, with a 1% involvement of world production. In 2019, milk production in the country was 7,184 million liters of raw milk (Fedegán, 2020).

Cattle are susceptible to being attacked by mites, viruses, fungi, bacteria, and nematodes, which decrease meat and milk production because they can affect cattle health (Parra *et al.,* 2013; Rutz *et al.,* 2015; Benavides *et al.,* 2016; Cadavid *et al.,* 2018; Giraldo *et al.,* 2011). Ticks belong to the Acari subclass, which is important ectoparasites in public and animal health. Their infestations cause great economic losses, not only by the depreciation of cattle hide but also by the decrease in production since they are vectors of pathogenic agents and their management increases production costs (Benavides-Montaño *et al.,* 2018).

Polanco-Echeverry *et al.* (2016) state that ticks are taxonomically located in the class Arachnida whose main characteristic is that in the adult stage, they have four pairs of legs and their body is divided into two regions, cephalothorax and abdomen. Hard ticks are organisms that require exclusively feeding on tissue and blood fluids to develop during all their stages and are classified as hemimetabolous arthropods.

A. cajennense transmits pathogenic microorganisms that cause diseases such as babesiosis or tick fever, which is characterized by causing erythrocyte lysis resulting in multiple health complications. In addition to its hematophagous action and painful bite, this tick species is known to act as a vector of spotted fever in Mexico, Panama, Colombia, and Brazil (Fuentes *et al.,* 2015). Similarly, it can affect different organisms such as cattle (*Bos taurus*), horses (*Equus caballus*), canines (*Canis lupus familiaris*), swine (*Sus scrofa*), capybaras (*Hydrochoerus hydrochaeri*), guinea pigs (*Cavia porcellus*), humans (*Homo sapiens sapiens*) and mules (*E. africanus x ferus*) (Meléndez *et al.*, 2014; Benavides *et al.,* 2011; Aguilar *et al.,* 2010). Its dissemination can become exponential, causing problems even at the public health level if its populations are not adequately managed (Robayo-Ortiz *et al.,* 2020).

For the management of hard ticks, chemical compounds based on ixodicides and macrocyclic lactones are commonly used in Latin American livestock farming, which, despite being effective, resistant populations have appeared due to the indiscriminate use of these products by producers (Intagri, 2019). This problem has prompted the implementation of cattle farms under organic parameters, which are based on sustainable animal production. This implies using agricultural techniques, livestock and materials that conserve and renew land resources, reduce pollution and erosion, promote the healthy and diverse development of theagricultural ecosystem and support pest control in a natural way (Toro *et al.,* 2012).

In the tropics and subtropics, the tick *Boophilus microplus* has shown resistance to chemicals such as chlorfenfinvos, coumaphos, diazinon, lindane, cypermethrin, deltamethrin, and flumethrin (Alonso-Díaz *et al.,* 2006). The most severe resistance occurs in the permethrin group, with rates above 47%. In the case of organophosphates, resistance is less than 31%. The least effective tickicide is cypermethrin, with a resistance of over 85% (González, 2003).

Calcium polysulfide, which is obtained by the thermal treatment of sulfur and lime mainly known for its fungicidal action (Tweedy, 1967; Smilanick *et al.,* 2001; Holb *et al*., 2003;Montag *et al*., 2005) and it is also used as an acaricide and insecticide (Guerra, 1985; Penteado, 2000; Guirado, 2001; Soto-Giraldo *et al.,* 2013; Navarro & Méndez, 2014; González *et al.,* 2011*;* Cabrera-Marulanda *et al*, 2018), it is also accepted by most organic certifiers (Venzon *et al*., 2008). The toxic effect of calcium polysulfide on insects and mites is given by the reaction of the applied product with water and carbon dioxide gas, resulting in hydrogen sulfide gas and colloidal sulfur (Abbot, 1945). This product has been successfully used for the control of the mite *Tetranychus urticae* (Acari: Tetranychidae), coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae), *Diaphorina citri* (Hemiptera: Liviidae), among others (Soto *et al*., 2013; Cabrera-Marulanda *et al.*, 2018; Restrepo *et al*.,2017).

The lethal dose (LD) is used to evaluate the toxicity of pesticides to arthropods (Sato *et al*., 2002; Stark *et al.,* 2003). However, LD is an incomplete measure of the effects of products on populations, as it analyzes only mortality as a toxicity parameter (Stark *et al.,* 2003; Peña *et al.,* 2013). The parameters that define the lethal are the concentrations in the active ingredients used. The objective of this work was to evaluatethe lethal and sublethal effects of calcium polysulfide on the *A. cajennense* tick, in reason to contribute to the rational management of the pest.

MATERIALS AND METHODS

The study was conducted at the Center for Research and Rearing of Natural Enemies of the University of Caldas, located at 05°03'N and 75°29'W (Manizales, Caldas). Nymphs of *A. cajennense* were collected in areas used for cattle raising at the Montelindo farm, Palestina municipality, Colombia, with an average temperature of 22.8°C. Species were determined by stereoscopic observations and comparisons of morphological guides and reference documents for ticks, as identification keys are visible at the base of the sub-ectangular capitulum accompanied its long slender palps. An ornamented shield scutum light brown, with patches forming a symmetrical pattern on the Coxa I present 2 widely separated spurs, long, narrow and triangular (Navarrete *et al.,* 2014; Voltzit, 2007). The lethal effect of calcium polysulfide on *A. cajennense* was determined by following the methodology described by Penteado (2000). Thus, the thermal treatment of sulfur and lime was carried out, using 250g of sulfur and 125g of lime for each liter of water. The concentration of calcium polysulfide obtained was 31.5° Baumé.

The lethal doses (LD) of calcium polysulfide were established through preliminary tests and were located between the lower limit, where the product did not cause mortality, and the upper limit of response, where it generated 100% mortality (Table 1). Ten individuals of *A. cajennense* were placed in Petri dishes of 9cm in diameter; the product was then sprayed with a manual atomizer.
The average volume of calcium polysulfide used in each spray was 1.3mL, equivalent to a deposit of 0.88±0.07mg/cm^2 on the treated surface. This applied quantity follows IOBC/WPRS recommendations (Overmeer & Van Zon, 1982) and represents the same characteristics as a field application (Reis *et al*., 1998). The control or control treatment was sprayed with distilled water following the same procedure. Finally, the Petri dishes were sealed and placed in a scientific incubator at 23±2°C and 60±10% relative humidity. The number of replicates per concentration in this preliminary assay was four.

With the results obtained in the initial trial, four concentrations of calcium polysulfide were selected for the final bioassay, ranging from 0.7% to 1.3% (Table 2). It was performed using the same methodology described in the preliminary trial with four replicates per concentration. Mortality was evaluated 24, 48, and 72h after the product application in the preliminary trial and in the final bioassay, at 96h after application (the time at which mortality is highest according to the preliminary tests). For each data set, standard and logistic distributions were assessed, then the models were evaluated using Proc Probit of the Statistical Analysis System (SAS) Enterprise Guide 8.3 statistical program.

Table 1. Concentrations of calcium polysulfide used in the bioassays on Nymphs of *A. cajennense.*

Bioassay	Treatment	Calcium Polysulfide Concentration (%)
Preliminary bioassay	Control	0
	1	0.5
	2	1.5
	3	2.5
	4	4
Final bioassay	Control	0
	1	0.7
	2	0.9
	3	1.1
	4	1.3

RESULTS AND DISCUSSION

In the evaluation of the toxicity of calcium polysulfide on *A. cajennense* (Table 2), significant results were observed 48h after application, where the statistical model produced a significant P<0.01 Chi-square test, so it was adjusted to a logistic distribution, described by the following equation:

$$\mathbf{Pr(response) = 0.1981(C)+0.8019(B)(\Phi(3.5432-26.6591\times log_{10}(dose)))}$$

Where parameter C is the natural threshold response or the proportion of individuals responding to the zero dose, which was estimated to be 0.1981; parameter B corresponds to the proportion of individuals who died by the action of the dose and Φ is the logistic distribution function.

According to the Probit Analysis, Cl50 corresponded to 1.05% and Cl_{95} to 1.35% concentrationof calcium polysulfide at 31.5°B (Table 2).

According to Table 2 the calcium polysulfide has acaricidal potential against *A. cajennense* ticks and could be used to control populations of this pest. However, the efficiency of alternative pest control products, such as calcium polysulfide, as well as the selectivity to natural enemies, is related to the dose and formulation used (Soto, 2010).

For the immature stages of *A. cajennense* to present 95% mortality, the concentration of calcium polysulfide is 1.35%, demonstrating that this product has the potential to be used in integrated management plans for ticks, which are currently considered a major pest in several countries.

Rivera *et al*. (2014) obtained mortality of *A. cajennense* higher than 90% with calcium polysulfide at 20% in volume. However, with the results obtained in the present research, it was possible to reduce the concentration of the product, causing the same effect on ticks, generating lower costs of raw materials in its preparation. Additionally, this dose is adjusted to the lethal concentrations recommended, and used in organic production systems. There they oscillate between 2 to 4% of the product with a density of 29 to 32°Baumé, applying approximately 0.58 to 1.28% of calcium polysulfide in the product (Penteado, 2000; D'andrea, 2001). The application of this product for ticks control contributes to livestock systems that wish to produce meat and milk under an ecological model, where the exposure of personnel and animals to toxins is minimized.

Peña *et al.* (2013) evaluated calcium polysulfide on *Aphis gossypii* (Hemiptera: Aphididae), finding 50 and 95% mortality in the population with a product concentration of 0.25 and 0.64%, respectively. In the management of the Asian psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), Restrepo *et al.* (2017) found that the lethal and sublethal doses of calcium polysulfide were 0.57 and 0.38%, respectively, demonstrating their effectiveness for pest control. Sosa *et al.* (2008) evaluated the effect of calcium polysulfide in a mixture with *Beauveria bassiana*, *Metarhizium anisopliae,* and EM-5 (mixture of microorganisms), resulting in the reduction of *Dysmicoccus brevipes* (Hemiptera: Pseudococcidae) populations in pineapple crops. In evaluations carried out

Table 2. Toxicity of calcium polysulfide in Nymphs of *A. cajennense*.

Product	Distribution	Cl 50[1] (C.I. at 95%)	Cl 95[2] (C.I. at 95%)	X^2	P
Calcium polysulfide	Logistics	1.05% (0.82 – 1.13)	1.35% (1.27 – 1.60)	12.4927	0.0004

[1)]Mean effective dose and 95% confidence intervals;[2)] Lethal effective dose causing 95%mortality and 95% confidence intervals.

by Zapata *et al.* (2011) with the application of deltamethrin (10mL of Deltamethrin 2.5% EC) on adults of *A. cajennense,* they found mortality of 72.6% at 24h after application. Da Silva *et al*. (2011) evaluated thymol extract (essential oils of thyme (*Thymus vulgaris*) or oregano (*Origanum majorana*)) on nymphs and adults of *A. cajennense*, finding nymph's mortality between 93.5 and 97.6%, and of adults of 100%. Anholeto *et al*. (2017) evaluated the effect of ethanolic extract of *Acmella oleracea* (Asteraceae) on *A. cajennense,* finding 100% mortality at 72h after application. Clemente *et al*. (2010) analyzed the lethal effects of essential oils of *Eucalyptus citriodora* and *Cymbopogon nardus* on *A. cajennense,* finding mortality between 53.1 and 61.1%. Cabrera *et al.* (2019) evaluated the mixture of calcium polysulfide with neem extract (*Azadirachta indica*) and black wood (*Gliricidia sepium*) and eucalyptus (*Eucalyptus*spp) in the bath of cattle on ticks, finding a 60% decrease of ticks.

With the results obtained in the present investigation, we found that calcium polysulfide with a concentration of 1.35%, has a mortality rate higher than 90% at 48h of application on nymphs of the specie *A. cajennense*, which constitutes an alternative to treatments with chemical products such as deltamethrin and cypermethrin, in addition to acting more efficiently with respect to plant extracts previously mentioned for tick management.

On the other hand, calcium polysulfide as a product for the management of insect pest populations can be associated with other biological inputs such as fungi and plant extracts, thus generating an alternative to the agrochemicals used in conventional management plans.

CONCLUSION

The use of calcium polysulfide in vitro tests represents a viable alternative to synthetic pesticides in the control of *A. cajennense*, due to the fact that at minimum concentrations, significant mortality of the pest is evidenced, so this input has the potential to be used in integrated control plans of the pest in question, thus reducing dependence on synthetic tickicide.

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