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# Effect of coumaphos on *Rhipicephalus microplus* and entomopathogenic nematodes in cattle production units

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# ABSTRACT

**Objective**: to determine the effect of coumaphos on *Rhipicephalus microplus* and entomopathogenic nematodes in bovine production units.

**Design/methodology/approach**: Two experiments were carried out: First) tick samples were collected and evaluated, using the Adult Immersion Test (10 ticks per humid chamber), the treatments were applied: 1: Control; 2: Coumaphos 0.1%; 3: Coumaphos 0.2%; 4: Coumaphos 0.4%. An ANOVA was carried out with a completely randomized design (mortality dependent variable and treatment independent variable with four levels) and a multiple comparison of means (Tukey's test). Second experiment) 10 samples of livestock soil where coumaphos is used, which correspond to the Mariano Matamoros ejido, Tamaulipas, and 10 non-livestock soil from the Las Huastecas Experimental Field, were analyzed to determine the presence of entomopathogenic nematodes (EPN). a cross-frequency table between the sampled soils and the Chi-square test.

**Results**: Experiment 1: In counting the tick mortality percentages on the second day after application in group 1: control was 37.5%; 2: 72.5%; 3: 80%; 4: 92.5%, on day 7 and day 8 there was no difference with 90% and 100% mortality. Experiment 2: The soils positive to EPN were 35% Non-livestock and 25% Livestock.

**Limitations on study/implications**: it is important to make producers aware of the correct use of chemical products.

**Findings/conclusions**: *R. microplus* was susceptible to coumaphos in cattle production units. A greater presence of EPN was found in non-livestock soils, which may be related to the use of chemical products to control parasites.

Keywords: Tick, ixodicide, resistance, nematodes, soils.



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# INTRODUCTION

Ticks are arthropods belonging to the Arachnida class, which in their adult phase have four pairs of legs as a characteristic and their body is divided into two regions, cephalothorax (union of head and chest) and abdomen, these parasites are considered one of the most important sanitary factors that limit cattle farming in the tropics, worldwide they affect 80% of the cattle ranching in the herds. It should be noted that they are hematophagous ectoparasites that feed on the blood of their host during their life cycle (Polanco *et al.*, 2016).

Ticks mainly inhabit warm and humid weather, and cause losses in livestock production systems if not adequately controlled, ticks *Rhipicephalus microplus* is widely distributed worldwide and has a high negative economic impact for cattle industries (Hurtado and Giraldo-Ríos, 2018). In the southern region of Tamaulipas, herds are made up of native cattle and specialized breeds for meat production: Beefmaster, Simmental, Suiz-Bú, among others which are used for their ability to adapt to the tropical weather (Garay *et al.*, 2020). The main economic losses caused by ticks are due to decreased growth, skin damage, decline in meat and milk production, zoonotic disease transmission, increased costs of disease control and treatment, as well as reproductive problems in cattle (Vargas-Cuy *et al.*, 2019).

The use of dewormers to control ticks affects the fauna present in the soil, such as entomopathogenic nematodes (EPN) and other organisms (Ruiz-Negrete *et al.*, 2018); which constitute a group of importance in the biological control of insects (García *et al.*, 2008). EPN are transparent organisms with dorsoventral movement, unsegmented with bilateral symmetry, in their anatomy they have excretory, nervous, digestive, reproductive and muscular systems, but they don't have the respiratory and circulatory systems, these are mostly found on the ground and to continue with the life cycle it is necessary that they infest an insect (Rosales *et al.*, 2008).

EPN are lethal parasitic organisms and obligate only of insects, have a ubiquitous distribution and are used as biological control agents, founding a mutualistic relationship with bacteria of the Enterobacteriaceae family, which require this host to obtain protection and be able to transport from one insect to another (Stock and Goodrich, 2008). The two most important and studied families are Steinernematidae and Heterorhabditidae, these are associated with bacteria of the genera *Xenorhabdus* and *Photorhabdus*, respectively (Vashisth *et al.*, 2013). Different strains of EPN have been used as biological control agents for various pests, mainly in the agricultural sector for the protection of various crops (Parada *et al.*, 2019), and in pests of livestock production animals (Alves *et al.*, 2012), although it is necessary to deepen its study in the livestock sector, as well as the effect that the chemical products used for animals have on the populations of these nematodes (Negrete *et al.*, 2018).

It is important to increase the effectiveness of tick control, always considering reducing economic costs, and taking care of animal health (Barrón-Bravo *et al.*, 2020a), using strategies to reduce environmental impact on non-target populations such as EPN (Barrón-Bravo *et al.*, 2020b). Considering the above, the objective of this study was to determine the effect of coumaphos on *Rhipicephalus microplus* and entomopathogenic nematodes in bovine production units.

# MATERIALS AND METHODS

Two experiments were carried out during the months of August to December 2021, in the first experiment, tick samples were collected and evaluated from a cattle production unit (CPU) and was evaluated with coumaphos treatment at different concentrations. In the second experiment, samples of Livestock Soil (LS) were analyzed, corroborating the use of coumaphos for the control of parasites in the same CPU, Non-Livestock soil (NLS) samples were also collected, in which coumaphos is not used, collected at INIFAP, Las Huastecas Experimental Field located in Villa Cuauhtémoc, Altamira, Tamaulipas, Mexico, to determine the presence of EPN.

## Location of the study area

The study was conducted at two locations: The first place was the private CPU that is located in the community Mariano Matamoros, Altamira, Tamaulipas (22° 39' 18.6" N and 98° 09' 47.5" W). The CPU aims to produce beef cattle in the cow-calf system, in extensive grazing, the herd has 12 breeding cows. The second place was the National Institute of Forestry, Agriculture and Livestock Research (INIFAP), Las Huastecas Experimental Field (22° 33' 57.2" N and 98° 09' 52.3" W), where is the Animal Health Laboratory located, in which the samples were worked, both locations are from the same municipality. Altamira has a warm sub-humid weather with rains in summer of medium humidity, an altitude between 0 and 600 m. Its temperature range is from 22 to 26 °C, its annual precipitation is from 900 to 1100 mm (Vargas *et al.*, 2007; INEGI, 2009).

## First experiment: Susceptibility of Rhipicephalus microplus to coumaphos

The Adult Immersion Test was used for this experiment (Drummond *et al.*,1973), four treatments were evaluated: 1: Distilled water (control); 2: coumaphos diluted 0.1%; 3: coumaphos diluted 0.2% y 4: coumaphos diluted 0.4%, performing four repetitions per treatment, with 10 ticks each one of them.

Adult ticks were collected directly from the body of the cows, carefully not to leave the mouthparts on the skin of the cattle. To do this, with the help of dissecting forceps, gently grasped the body of the ticks, he turned on his back and then quickly pulled in a direction perpendicular to the skin. 10 ticks were grouped and placed in petri dishes with wet filter paper, a total of 160 ticks were tested, they were transported in an expanded polystyrene cooler at a temperature of  $25\pm2$  °C to the Animal Health Laboratory. After 24 h the treatments were applied, it started with the control group, first with the lowest concentration and ending with the highest concentration. 50 mL of distilled water were added for the control group and the same volume for the ixodicide of each treatment, in the beakers they were kept immersed for 30 s.

After the immersion time, filter paper was placed to extract the ticks from the solutions and they were returned to the petri dish with wet filter paper, finally, they were labeled for identification. Petri dishes were incubated at a temperature of  $25 \pm 2$  °C and 80 to 90% relative humidity. Tick mortality was assessed daily, considering the day after application (DAA) from 1 to 6 to register the result in a database (Drummond *et al.*, 1973; Jonsson *et al.*, 2007).

# Second experiment: Determination of the presence of entomopathogenic nematodes in livestock soils and non-livestock soils

Soil samples were taken using the five-gold technique. Samples of LS in the CPU from the Mariano Matamoros community and samples of NLS from Las Huastecas Experimental Field, INIFAP. The samples were transported in an expanded polystyrene cooler to the laboratory at  $25\pm2$  °C (López-Llano and Soto-Giraldo, 2016). For the isolation of entomopathogenic nematodes, the trap insect methodology was used, described by Bedding (1984) and Kaya and Stock (1997), the one kg soil samples were sieved, moistened and placed in a plastic container with a capacity of 1,000 mL, and were added 10 larvae of Tenebrio molitor in samples collected from the area LS and NLS, they were left for seven days in the dark, at a temperature of  $25\pm2$  °C. After this time, the dead larvae or pupae were transferred to a petri dish with wet filter paper. It was observed daily for 7 days under the microscope, once identifying the infection by EPN on the dead larva, transferred to a White Trap moistening with distilled water for later identification (Nguyen and Smart, 1996).

#### Analysis

Tick mortality data (dependent variable) were analyzed with the PROC GLM (SAS, 2002), under a completely randomized design, with four replications. The independent variables were the treatments and DAA. The comparison of means was performed using the Tukey test ( $\alpha$ =0.05). Regarding the results of entomopathogenic nematodes, they were organized in a database in the Microsoft Excel Program, Subsequently, the general prevalence was determined. A cross-frequency table was made between the sampled soils and the Chi-square test was performed, in the statistical program Statgraphics, edition 18 (2017).

#### **RESULTS AND DISCUSSION**

The effect of coumaphos on *R. microplus* and entomopathogenic nematodes in cattle production units was determined, the results are shown in the first and second experiment below:

#### First experiment: Susceptibility of Rhipicephalus microplus to coumaphos

It was observed that from the day 1 DAA, mortality was greater than a 60 % (Table 1). No differences were observed between the different concentrations of coumaphos that were used (P>0.05). 100% mortality was observed in the 4th DDA at 0.20% of coumaphos. With the lowest concentration of coumaphos (0.05%), a 100% mortality was obtained on day 6th DAA (Table 1).

Growing tick populations are a serious problem worldwide, as well as the resistance to the products used for its control, the results show how these methodologies strengthen control programs and can be used anywhere this problem occurs, Bravo *et al.* (2008) conducted an experiment on *R. microplus* ticks collected from four dairy farms, its susceptibility did not present significant differences between the groups treated with the five concentrations evaluated in each farm; the increase in coumaphos concentration was

1								
	Days after application							
Coumaphos Treatment (%)	1	2	3	4	5	6		
	Mortality (%)							
Control	22.5 b	37.5 b	37.5 b	40.0 b	42.0 b	45.0 b		
0.1	60.0 a	72.5 ab	80.0 a	90.0 a	95.0 a	100.0 a		
0.2	62.5 a	80.0 ab	95.0 a	95.0 a	100.0 a	100.0 a		
0.4	67.5 a	92.5 a	97.5 a	100.0 a	100.0 a	100.0 a		
P-Value	0.0224	0.0212	0.0013	0.0024	0.0003	0.0002		

Table 1. Susceptibility of Rhipicephalus microplus to different concentrations of coumaphos.

Different literals between lines indicate significant difference (Tukey;  $\alpha = 0.05$ ).

not directly proportional to the efficacy. *R. microplus* adults were resistant; however, 100% mortality was observed in larvae, which indicates high susceptibility of the larvae to this organophosphate, Similar results to the present study where a 45% mortality of the control group was obtained and in the concentrations that contained coumaphos 100% being susceptible.

Reyes-Domínguez *et al.* (2013) evaluated the resistance of *R. microplus* to coumaphos from San Juan Evangelista, Veracruz, Mexico, they worked in 16 CPU dedicated to the dual-purpose system (meat and milk). In each CPU, 50 female *R. microplus* ticks were collected to determine resistance to coumaphos, deltamethrin and amitraz, using the adult immersion test. In the CPUs sampled, populations of ticks with multiple resistance to organophosphates, synthetic pyrethroids and amitraz were identified, however, 2 of 16 did not present resistance to organophosphates. There are differences with the present study, since in the studied CPU a 100% mortality was obtained with the organophosphate coumaphos, which may be due to the different management in the dual-purpose production system in which regularly the control of ectoparasites is more frequent.

Rodríguez-Pacheco *et al.* (2017) evaluated the *in vitro* resistance of the *R. microplus* to organophosphates, synthetic pyrethroids and amitraz. Adult ticks were collected in Boyacá, Colombia, and they were subjected to the adult immersion test to evaluate the efficacy of the ixodicides, the effect on oviposition and the percentage of larval hatching. Their results demonstrated mortality rates of 43% in the control, 56% synthetic pyrethroids, 60% amitraz and 96% organophosphates, at 21 days DAA. The oviposition inhibition was 13.4% for the control, 44.6% for amitraz, 45.5% for synthetic pyrethroids and 96% for organophosphates. The hatching percentage of eggs was 88% for the control, 16% for amitraz, 14% for synthetic pyrethroids and 4% for organophosphates, there was a significant difference in the organophosphate group. Their results agree with those of the present study, showing high mortality when using organophosphates, which may be since dewormers are rotated to make efficient use.

Coronado and Mujica (1997) studied the adult females of R. microplus, which they collected from cattle on farms in eight states of Venezuela, using the adult immersion test (Drummond test). The acaricides used were: coumaphos, chlorfenvinphos, amitraz, iminothiazole, cypermethrin and flumethrin, respecting the doses recommended by the

manufacturers. The results obtained show variable levels of resistance according to the area of origin of the ticks. The highest levels of resistance were observed in organophosphates and in cypermethrin. Their results differ from those of the present study, where susceptibility on the part of the parasite is observed, most likely due to the indiscriminate use mentioned by the authors.

Rentería and Sevilla (2015) conducted an experiment in five cattle ranches in the state of Nuevo León (Terrabel, Bisonte, El Pájaro, Valle Escondido and 14 de Mayo), collected tick samples from cattle *R. microplus*, because the producers showed concern about diseases and economic losses caused by these ectoparasites, and to diagnose susceptibility to the products used in its control. Organochlorines (Lindane), organophosphates (Coumaphos, diazinon, chlorfenvinphos) and pyrethroids (Flumethrin, deltamethrin and cypermethrin) were used. Their results showed a greater susceptibility to Deltamethrin, followed by chlorfenvinphos, diazinon, flumethrin, cypermethrin, lindane and lower mortality for coumaphos, which is the most common product in tick control. Its results are similar to those obtained in the present study due to the common use of coumaphos in the CPU of both regions, so integral management methods for tick control should be suggested.

# Second experiment: Determination of the presence of entomopathogenic nematodes in livestock soils and non-livestock soils

The presence of EPN in the LS was 25% positive and 25% negative, in contrast to the results for NLS with 35% positive and 15% negative of the total of 20 (100%) samples (Table 2), the EPN found were identified as belonging to the Steinernematidae family.

The issue of EPN has various applications, they are beneficial organisms for the agricultural and environmental sector, so their study is of great importance in order to make the most of their potential worldwide, Montoussé *et al.* (2008) investigated the presence of EPN in chestnut orchards *Castanea sativa* Mill (Fagaceae) from Galicia, España, since they are of interest to start the biological control program of the chestnut plague *Curculio elephas* Gyllenhal (Coleóptera: Curculionidae) and butterflies of the genus *Cydia* Hubner (Lepidoptera: Pyralidae), these pests cause severe damage. They collected samples in 30 characteristic points of the provinces Pontevedra and Ourense, analyzing the EPN using the insect trap technique with larvae of *Galleria mellonella* L. (Pyralidae). They were identified by morphological and morphometric analysis and by molecular techniques, the nematodes of the genus *Steinernema* and *Heterorhabditis*, in four of the sampled locations.

<b>EDN</b>	Soil ty	T-+-1 (0/)		
EPN presence (%)	Not livestock	Livestock	Total (%)	
EPN negative	3 (15.0)	5 (25.0)	8 (40.0)	
EPN positive	7 (35.0) <sup>a</sup>	5 (25.0) <sup>b</sup>	12 (60.0)	
Total	10 (50.0)	10 (50.0)	20 (100.0)	

**Table 2**. Presence of entomopathogenic nematodes (EPN) in relation to the type of soil in Altamira Tamaulipas, Mexico.

Different literal by column indicates significant differences (Chi-square test);  $\alpha = 0.05$ .

The results were positive, since there is a presence of EPN in four of the 30 places analyzed, with an abundance of 13.3%. The presence of EPN in the soil varies due to numerous factors, not only physical such as texture, humidity, temperature, aeration or soil pH, but also by biological factors such as the abundance of host insects in the environment. Their results show lower percentages than those of the present study in terms of the presence of EPN in LS and NLS, which may be due to the different climatic conditions and the chemical products used in the management of these places.

Parada *et al.* (2006) They located zones that correspond to the area of greatest production of potato *Solanum tuberosum* L. (Solanaceae) in 24 municipalities of Colombia, where they collected insects whose movements were very slow, or corpses of yellow to dark cream coloration, of a soft consistency, but not rotten, in addition to soil samples to carry out the insect trap technique, with a total of 288 places, each sampling point comprising five subsamples, which were taken with a shovel at a depth of 25 and 35 cm, collecting 300 g of soil. Infected insects in the isolation of nematodes that were arranged in wet petri dishes and White traps. Their results show 56 % of positive soils to EPN, in addition to 100% of the nematodes that were associated with the collected insects, 55% of the population corresponded to species of the *Steinernematidae* family and the remaining 45% were located within the order Rhabditida, mainly Rhabditonematidae and Rhabditidae families. In the present study, the same insect trap technique was carried out, obtaining results below the percentage of EPN presence of this study, which is surely due to the richness in organic matter of the soils, as well as the favorable conditions for the EPN of the sampled points.

Molina-Acevedo et al. (2006) conducted an experiment in the State of Minas Gerais, Brasil, with the objective of determining the percentage of survival in storage of six species of EPN, Steinernemátidos (Steinernema carpocapsae Weiser, Steinernema glaseri (Steiner) Wouts, Mracek, Gerdin & Bedding and Sedum arenarium Brotero (Rhabditida: Steinernematidae)) and three Heterorhabdítidos (Heterorhabditis bacteriophora Poinar, H. bacteriophora HP88 and Heterorhabditis baujardi LPP7 Weiser (Rhabditida: Heterorhabditidae)), at five different temperatures (8, 12, 16, 20 and 24 °C), with two concentrations (1000 and 10000 Infective Juveniles/mL) in a period of 15 days and three months. Most of the Steinernematids gradually increased in a wide temperature range from 8 to 20 °C, in both concentrations and in the shortest time, registering survival between 87 and 95%. On the contrary, high temperatures 20 and 24 °C, together with the lowest concentration and time, favored the high survival of Heterorhabditids, being between 78 and 92% respectively. Thus, in this experiment it was possible to determine specific conditions for each EPN, which represents a high survival. Their results are similar in the EPN management conditions of the present study, although the objectives differ, the differences observed based on genera may be mainly due to the different EPN species of each place and their adaptation.

Mercedes-Lucero et al. (2006) conducted a study in Colombia, with the objective of evaluating the use of entomopathogenic microorganisms (*Beauveria bassiana* (Balsamo) Vuillemin (Cordycipitaceae), *Metarhizium anisopliae* (Mechnikov) Sorokin (Clavicipitaceae) and *Steinernema* sp. in pest insect control chiza (*Astaena* sp.), as well as the agroecological sustainability of wheat and potato production systems on smallholding farms in the municipalities of Ospina and Yacuanquer in the Department of Nariño. The experiments

were carried out under greenhouse and field conditions on producer farms, located at an altitude between 2,650 and 2,850 m, with an average temperature of 12 °C, relative humidity between 75% and 80%, average rainfall of 800 to 1000 mm.

The application of entomopathogens was carried out using decomposed wheat chaff in a ratio of 1:10. With the application of entomopathogenic fungi, they achieved the percentage mortality of the pest with 28.75% (in Ospina) and 14.67% (in Yacuanquer), for chlorpyrifos there was a percentage mortality of 28.89% (Ospina) and 18.82%. (Yacuanquer). With the Steinernema nematode, a mortality of 17.27% (Ospina) and 12.74% (Yacuanquer) was observed. The application of a mixture of entomopathogenic microorganisms and decomposed wheat chaff to the crops contributed to reduce environmental contamination and to improve the characteristics of the soils. Their results, although they differ from the present study because only the presence of entomopathogenic nematodes was evaluated, show the practical application that can be developed in control programs based on entomopathogens and the need to study their potential.

Méndez-Barceló et al. (2011) carried out a study to identify EPN in Colombia, in the towns of Buenaventura, Valle del Cauca, (Cisneros, Córdova and Llano Bajo), polyculture areas of chontaduro (*Bactris gasipaes* H. B. K.), papachina (*Coloasia esculenta*, Schott), banana (*Musa* sp.), árbol del pan (*Arthocarpus* sp.), yuca (*Manihot sculenta*, Krantz), borojó (Borojoa patiñoi, Cuatr.) and fruit and forest species. The weather of these areas is humid tropical forest with an average annual temperature of 28 °C and rainfall of 7000 mm per year. For the isolation, the trap insect technique was used. The EPN that were found were inoculated again into *G. mellonella* larvae in order to evaluate their pathogenicity and obtain samples for identification at the genus level. They detected EPN in 36.84% of the samples, identifying only the genus Steinernema in the three locations, mainly where the decomposing peach tree is found, which has a significant incidence of these organisms in traditional cropping systems in the rural area of Buenaventura. The isolated nematodes caused 100% mortality in *G. mellonella* larvae at 48 h. Their results are similar to the present study, because they found the same EPN family, and a very similar percentage to those of NLS, this may be due to the similarity in the conditions of the sampled places.

In the study of Barron *et al.* (2020b) simples from LS and NLS were analyzed, collected from the municipalities of Irapuato and León, Guanajuato, México, from which were isolated EPN from Steinernematidae and Heterorhabditidae family. A 37% of positive samples were obtained at EPN, which most were LS with 24.3% and in smaller quantity NLS with 13.5%. These results differ from those of the present study where a greater presence was observed in NLS, which is probably related to the use of chemical products related to parasite control, which is more frequent due to the favorable conditions of humidity and temperature for the development of parasites in this area, especially tick, LS LS are rich in organic matter and present better conditions for EPN, but this is affected by the indiscriminate use of chemical products related to pest control.

## CONCLUSIONS

*Rhipicephalus microplus* was susceptible to coumaphos in the bovine production unit in southern Tamaulipas, the methodologies used can strengthen control programs, continuous

monitoring of ixodicides is very important to detect resistance, emphasizing management training Integrated tick adapted to cattle production systems. The effect of coumaphos on the populations of entomopathogenic nematodes, was manifested whit a greater presence was found in non-livestock soils, which may be related to the recurrent use of chemical products to control parasites in livestock soils from cattle production units and the growing populations of ticks in the area, which forces the repetitive and indiscriminate use of ixodicides, affecting populations of beneficial organisms which are not their objective.

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