v. 4, n. 7, 2024

International Journal of Biological and Natural Sciences

Acceptance date: 22/11/2024

ALTERNATIVES FOR THE CONTROL OF THE TICK *Rhipicephalus* (Boophilus) *microplus* BASED ON *Bacillus thuringiensis*

Caleb C. Lormendez
Center for Biological Research, Autonomous University of the State of Morelos, Mexico
Cuernavaca, Morelos, Mexico
https://orcid.org/0000-0002-3068-0508

Mayra Cobaxin-Cárdenas
National Center for Disciplinary Research in Animal Health and Food Safety, INIFAP
Jiutepec, Morelos, Mexico
https://orcid.org/0000-0001-9984-2715

Erubiel Toledo-Hernandez
School of Chemical and Biological Sciences, Autonomous University of Guerrero
Chilpancingo, Guerrero, Mexico
https://orcid.org/0000-0003-4211-5298

César Sotelo Leyva
School of Chemical and Biological Sciences, Autonomous University of Guerrero
Chilpancingo, Guerrero, Mexico
https://orcid.org/0000-0002-4710-4590

Guadalupe Peña Chora
Center for Biological Research, Autonomous University of the State of Morelos
Cuernavaca, Morelos, Mexico
https://orcid.org/0000-0001-7682-5584

Abstract: Microbial control of the tick *Rhipicephalus microplus* has focused on evaluating toxicity to adults and larvae, with a response time varying from 3 to 10 days to cause mortality in ingested ticks, depending on the species of bacteria used. However, during this period, ticks may oviposit as a defense mechanism. Therefore, the objective of this work was to evaluate the ixodicidal effect of the spore-crystal complex of *Bacillus thuringiensis* strain GP543 for the control of *R. microplus* ticks. Mortality and hatching inhibition percentages were evaluated in immersion bioassays with freshly ingested female ticks with concentrations of 50 and 300 μg-mL^{-1} of the spore-crystal solution and distilled water as a control. The bioassay was performed under a completely randomized experimental design, each experimental unit contained 24 individuals, individually distributed in 24-well cell culture plates, with 4 replicates. The highest percentage of mortality in the treated ticks was recorded at 14 days, at this time a mortality of 51.7% was observed at the lowest concentration used (50 μg-mL^{-1}) and a higher percentage of mortality (75.0%) when the concentration of the spore-crystal complex was increased to 300 μg-mL^{-1} . Regarding the effect on hatching inhibition, values of 85.2% (50 μg-mL^{-1}) and 89.2% (300 μg-mL^{-1}) were obtained, which, although statistically equal, are significant values. These results demonstrate that the spore-crystal complex of *B. thuringiensis* strain GP543 is able to reduce oviposition and hatching of ixodicide-resistant ticks.

Keywords: microbial control, spore-crystal complex, ovicidal effect, cattle tick.

INTRODUCTION

Mexico is among the main beef producing countries; in 2023 it ranked sixth worldwide as a beef producer (COMECARNE, 2023), however, the cattle industry faces constant threats that affect the health of cattle. Among these threats is the tick *Riphicephalus microplus*, which has become a major concern for the cattle industry due to the damage caused by infestation by this tick, since due to the large amount of blood sucked from cattle it impacts milk and meat production, damages the hide, causes toxicity and leads to systemic hemostatic changes resulting from the inoculation of substances present in the tick's saliva (Beys-da-Silva et al., 2020). Also, because of the diseases they transmit such as babesiosis (caused by protozoa *Babesia spp*) and anaplasmosis (caused by the intraerythrocytic bacterium *Anaplasma marginale*) (Beys-da-Silva et al., 2020). Overall, the damage caused translates into substantial economic losses; in 2017, annual losses were estimated at US$573 million, reflecting the impact of *R. microplus* on national cattle production (Rodríguez-Vivas et al., 2017).

The methods used to control *R. microplus* are diverse: mechanical, vaccines and chemical acaricides, among others. However, the massive and irrational use of chemical ixodicides has caused the appearance of strains resistant to one or several of these acaricides (Tabor et al., 2017, Almazán et al., 2010, Cooper 1974); in this regard, in Mexico, strains resistant to up to three chemical products have been reported. Added to this, the high residuality, bioaccumulation and high toxicity to non-target species, generate a high ecological impact. In this sense, the production of new active complexes for the control of organisms that have lost sensitivity to chemical products is of great interest. One of the alternatives is the use of the bacterium *Bacillus thuringiensis* (Bt), which has been

shown to be highly effective in the control of insect pests of agricultural importance, as well as insect vectors of human diseases. The objective of this work is to evaluate a bio-ixodicide (garrapaticide) based on the spore-crystal complex of Bt strain GP543, which synthesizes an S-layer protein called GP543-SL, with toxic activity against ticks.

MATERIALS AND METHODS

BIOLOGICAL MATERIAL

For the bioassays we used Bt strain GP543, collected in the field and stored in the collection of the Plant Parasitology Laboratory of the Biological Research Center, UAEM, and ingested adult female ticks of *R. microplus* strain Media Joya, which is susceptible to ixodicides, maintained under laboratory conditions at CENID-SAI-INIFAP in Jiutepec, Morelos, Mexico (Gaxiola-Camacho, et al., 2009).

IMMERSION BIOASSAYS

The ixodicidal effect of the Bt GP543-SL protein was evaluated by immersion bioassays of ingurgitated ticks, using two concentrations of the protein (50 and 300 μg-mL^{-1}) and distilled water was used as a negative control. For this purpose, 24-well plates (NUNC®) were used and 1 mL of the spore-crystal solution with the concentration required in the bioassay was placed in each well. Subsequently, one tick per well was placed and completely submerged in the corresponding suspension of the spore-crystal complex for five minutes, after which time the ticks were removed from the suspension and dried with absorbent paper to completely eliminate the suspension. Finally, ticks were individually placed in the same arrangement as they were in a clean 24-well plate and incubated for 14 days in a humid chamber at 90% relative humidity and 25 ± 2 °C (Drummond and Whetstone, 1970).

EXPERIMENTAL DESIGN AND ANALYSIS

Each experimental unit was formed with 24 ingested adult female ticks with four replicates. Ticks were homogenized by weight; for this purpose, each individual was weighed using an analytical balance (OHAUS AS 120) with the weights obtained we generated a graph (frequency versus weight) with a normal distribution, to discard the extremes of the distribution (heaviest and lightest ticks) and thus select a homogeneous sample. After this selection, ticks were completely randomly distributed in 24-well plates, numbered for subsequent identification. This experiment was replicated twice and mortality percentages were calculated at 8, 10, 12 and 14 days after treatment. A completely randomized experimental design was used and analyzed using Tukey's multiple range tests. SAS software (Version 9.0) was used for statistical analysis.

MORTALITY PERCENTAGE

From day 8, 10, 12 and 14 after immersion of the ticks in the treatments, dead ticks were counted. Mortality was determined by observation under a stereoscopic microscope, corroborating that the ticks did not show abdominal movement, even after applying tactile stimulation with a brush, remaining immobile until the end of the experiment.

OVIPOSITION INHIBITION PERCENTAGE (% O.I.)

The % O.I. was determined after 14 days of incubation. Eggs oviposited throughout the experiments were collected and weighed per experimental unit using an analytical balance, the % O.I. was determined according to the following equation: % O.I. = (PQLt/PQLT-PHLt/PHLT) *100

Where: PQLt = weight of female ticks in the experimental unit, PQLT = weight of female ticks in the control unit, PHLt = weight

International Journal of Biological and Natural Sciences ISSN 2764-1813 DOI https://doi.org/10.22533/at.ed.813472416109

of oviposited eggs in the experimental unit and PHLT = weight of oviposited eggs in the control unit. SAS software (Version 9.0) was used for statistical analysis.

RESULTS AND DISCUSSION

SELECTION OF A HOMOGENEOUS GROUP OF *Rhipicephalus microplus* SAMPLES BY WEIGHT

One of the important aspects to consider in the bioassays is that the mortality variable is sensitive to the effect of the weight of the individuals, so that the dose-mortality is influenced by the weight of the ticks. As expected, tick weights were heterogeneous, ranging from 15.5 to 371 mg in a sample of 615 individuals. Therefore, to reduce this variability in tick weights, a histogram of tick weight frequencies was generated. The replete females collected were weighed individually and a normal distribution was developed from the weight data (Table 1).

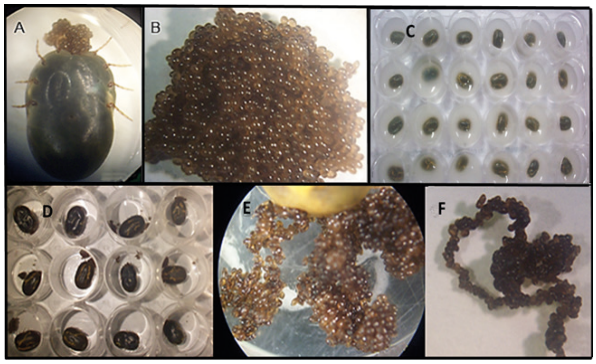
Ticks weighing less than 154 mg and more than 312 mg were removed from the bioassay, eliminating a total of 180 individuals from the initial sample of 615, obtaining a homogeneous block with 435 individuals with which 18 experimental units were formed.

For the immersion bioassays, the homogeneous sample was distributed in 24-well plates in a completely random manner. Table 1 shows the block that was eliminated and the homogeneous group, as well as the histogram of the total collection, which has a normal dispersion in weight (Figure 1).

The formation of homogeneous units (a generally neglected aspect) in the weight of *R. microplus* ticks made it possible to reduce the variability of mortality and its statistical validation, showing that even at low concentrations this ovicidal effect is rendered.

EVALUATION OF THE TOXIC ACTIVITY OF THE SPORE-CRYSTAL COMPLEX OF BT STRAIN GP543

Following the application of the treatments, a decrease in the percentage of O.I. in ticks was observed, as well as a decrease in the weight of oviposited eggs (Figure 2, panel D)), although the greatest impact observed was on the reproductive potential (% O.I.) (Figure 2, panel F) by inhibiting egg hatching in the treatments from a concentration of 50 µg-mL^{-1} of the spore-crystal complex (Table 2).



Effect of the pathogenicity of the spore-crystal complex of Bt GP543-SL with 50 µg-mL^{-1} on *R. microplus* at 14 days post-treatment: (A) control tick, (B) ovigerous mass from control tick, (C) tick immersion bioassay, (D) decreased oviposition weight in treated ticks, (E) oviposition of treated ticks, (F) ovicidal effect, newly oviposited eggs are observed dehydrated and infertile.

In both concentrations of the spore-crystal complex of Bt tested, a percentage of mortality greater than 50% was observed 14 days post-treatment, reaching the maximum percentage of mortality (75%) in ticks treated with 300 µg-mL^{-1} of the spore-crystal complex, at this same concentration and time a decrease in the oviposition rate of 5.2% and a viability of hatched eggs (% I.H) of 89.2% were also observed (Table 2). It is worth mentioning that in these last two parameters evaluated, no significant differences in oviposition rate and % I.H were observed at the concentrations

	Weight (mg)	Class	Limit lower (mg)	Upper limit (mg)	Media (mg)	Frequency (No. individuals)
Max. weight	410	1	**15.5**	55.00	35.25	3
Min. weight	15.5	2	55.00	94.50	74.75	9
Range	395	3	94.50	134.00	114.25	35
Class Numbers	10.3	4	134.00	173.50	153.75	81
	10	5	173.50	213.00	193.25	**142**
Class Interval	39.5	6	213.00	252.50	232.75	**173**
		7	252.50	292.00	272.25	**120**
		8	292.00	331.50	311.75	43
		9	331.50	**371**.00	351.25	9
		10	371.00	410.50	390.75	0
						615

Table 1 Table of weight distribution of *Rhipicephalus microplus*.

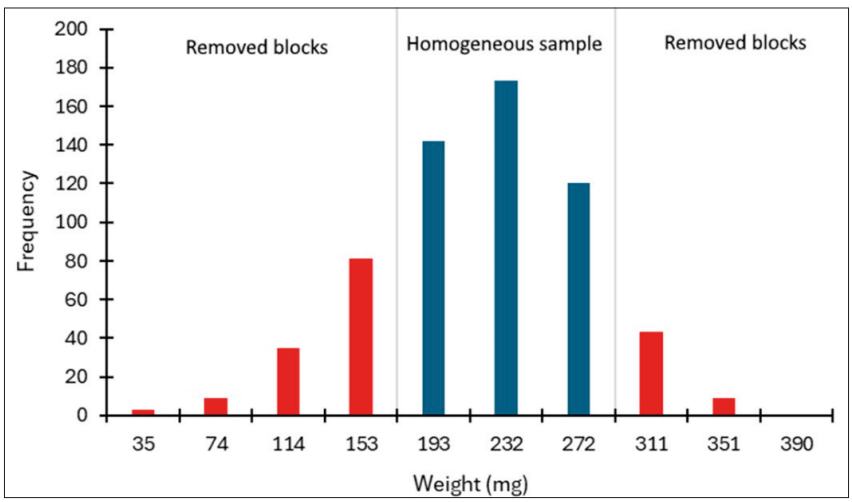


Figure 1. Histogram of the weight distribution of *Rhipicephalus microplus*.

Spore-crystal complex	Mortality rate post treatment				(% O.I.)	(% H.I.)
[µg-mL]$^{-1}$	8 days	10 days	12 days	14 days	14 days	14 days
300	22.2 ± 0.0 a	40.0 ± 0.0 a	62.2 ± 0.0 a	75.0 ± 4.8 a	5.2 ± 1.4 a	89.2 ± 3.8 a
50	23.1 ± 0.9 a	30.4 ± 0.6 b	39.1 ± 0.8 b	51.7 ± 2.0 b	4.8 ± 1-2 a	85.2 ± 7.2 a
H_2O	0.0 ± 0.0 b	2.7 ± 4.7 c	5.5 ± 3.6 c	10.2 ± 0.6 c	0.0 ± 0.0 b	8.7 ± 1.3 b

Table 2. Pathogenicity of the spore-crystal complex of *B. thuringiensis* strain GP543 against *R. microplus*.

Values represent the average of 4 replicates ± SD, values with the same letter have no significant differences, data were evaluated by Tukey's test with an α = 0.05.

tested (50 µg-mL and 300 µg-mL-1), suggesting that a concentration of 50 µg-mL of the spore-crystal complex may be sufficient to affect tick reproduction *in vitro*.

These results show the pathogenic action of the spore-crystal complex of Bt strain GP543 on ingurgitated females of *R. microplus* media joya being consistent with what has been reported (Lormendez et al., 2019). Likewise, by favoring mortality in females and inhibiting egg hatching, it suggests that the Bt spore-crystal complex could represent a new microbiological control option capable of affecting the biological cycle of *R. microplus* significantly, if we take into consideration that each replete female oviposits on average

3,670 eggs during an oviposition period of 12 days, with a hatching percentage around 78.1 % (Davey et al., 2006) and our results show a viability of 85.2% with 50 μg-mL^{-1} of the spore-crystal complex (Table 2). However, it is important to consider that, although the bioassays performed with the spore-crystal complex of *B. thuringiensis* show that its *in vitro* performance is favorable for the control of this ectoparasite in cattle, in *vivo* experiments in naturally infected cattle (field) will be necessary to evaluate its ixodicidal effect.

CONCLUSIONS

One of the main reasons for seeking new strategies for the control of *R. microplus* ticks is resistance to commercial ixodicides. In this regard, the results of this work suggest that the spore-crystal complex of Bt strain GP543 could be used as a bio-ixodicide for the control of resistant *R. microplus* strains, since it was not only able to inhibit oviposition, but also presented ovicidal effect.

Acknowledgments: The authors would like to thank Dr. Manuel Fernández Ruvalcaba of CENID-SAI, INIFAP, for his support in obtaining the ticks.

REFERENCES

Almazán, C., Lagunes, R., Villar, M., Canales, M., Rosario-Cruz, R., Jongejan, F., & de la Fuente, J. (2010). Identification and characterization of *Rhipicephalus* (Boophilus) *microplus* candidate protective antigens for the control of cattle tick infestations. *Parasitology research*, *106*, 471-479.

Beys-da-Silva, W. O., Rosa, R. L., Berger, M., Coutinho-Rodrigues, C. J., Vainstein, M. H., Schrank, A., & Santi, L. (2020). Updating the application *of Metarhizium anisopliae* to control cattle tick *Rhipicephalus microplus* (Acari: Ixodidae). *Experimental Parasitology*, *208*, 107812.

Consejo Mexicano de Carne https://comecarne.org/wp-content/uploads/2024/05/compendio-estadistico-2024-V2.pdf. Consultado el 20 de agosto del 2024.

Cooper Mc.D. Robertson L.T.D. (1974). Control de las garrapatas del ganado vacuno. Berkhamsted. 66-68.

Davey, R. B., George, J. E., & Miller, R. J. (2006). Comparison of the reproductive biology between acaricide-resistant and acaricide-susceptible *Rhipicephalus* (Boophilus) *microplus* (Acari: Ixodidae). Veterinary parasitology, 139(1-3), 211-220.

Drummond, R. O., & Whetstone, T. M. (1975). Oviposition of the Cayenne tick, *Amblyomma cajennense* (F.), in the laboratory. Annals of the Entomological Society of America, 68(2), 214-216.

Gaxiola-Camacho, S., García-Vázquez, Z., Cruz-Vázquez, C., Portillo-Loera, J., Vázquez-Peláez, C., Quintero-Martínez, M. T., & Rosario-Cruz, R. (2009). Comparison of efficiency and reproductive aptitude indexes between a reference and field strains of the cattle tick *Rhipicephalus (Boophilus) microplus*, in Sinaloa, Mexico. Revista Brasileira de Parasitologia Veterinária, 18, 9-13.

Lormendez, C. C., Fernandez-Ruvalcaba, M., Adames-Mancebo, M., Hernandez-Velazquez, V. M., Zuñiga-Navarrete, F., Flores-Ramirez, G., ... & Peña-Chora, G. (2019). Mass production of a S-layer protein of *Bacillus thuringiensis* and its toxicity to the cattle tick *Rhipicephalus microplus*. Scientific reports, 9(1), 17586.

Rodríguez-Vivas, R. I., Grisi, L., Pérez-de León, A. A., Silva-Villela, H., Torres-Acosta, J. F. J., & Fragoso-Sánchez, H. (2017). Evaluación del impacto económico potencial de los parásitos del ganado bovino en México. Revisión. Rev Mex Cienc Pecu, 8(1), 61-74.

Tabor, A. E., Ali, A., Rehman, G., Rocha Garcia, G., Zangirolamo, A. F., Malardo, T., & Jonsson, N. N. (2017). Cattle tick *Rhipicephalus microplus*-host interface: a review of resistant and susceptible host responses. Frontiers in Cellular and Infection Microbiology, 7, 506.