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Mortality of partially engorged *Rhipicephalus microplus* female ticks with a commercial product of *Beauveria bassiana* and *Metarhizium anisopliae*

Gabriela Alvarado-Rodríguez[1,2], Mara Cruz-González[1], Luis Moisés Camacho-Sosa[1], Jerónimo Herrera-Pérez, Vicente Homero González-Álvarez[1*]

[1] Facultad de Medicina Veterinaria y Zootecnia No. 2, Universidad Autónoma de Guerrero. Cuajinicuilapa, Guerrero, México.

[2] Doctorado en Producción y Sanidad Animal, Universidad del Mar. Puerto Escondido, Oaxaca, México.

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***Corresponding author:** Vicente Homero González-Álvarez

Abstract

In the present work, we evaluated the effect of a product formulated from the entomopathogenic fungi Beauveria bassiana and Metarhizium anisopliae on the mortality of partially engorged females of Rhipicephalus microplus ticks. Four concentrations (25%, 50%, 75%, and 100%) of the product were prepared diluted in distilled water, and immersion tests will be carried out on the specimens. Tick mortality of 80%, 60% and 50%, respectively, was observed at 96-, 120- and 144 hours post-exposure. It is necessary to carry out additional studies with fully engorged and to evaluate their effect as well as their ovicidal and larvicidal capacity.

Keywords: Entomopathogenic fungi, mortality, ticks.

INTRODUCTION

Rhipicephalus microplus ticks are common in cattle and the infestations cause economic losses (Lagunes-Quintanilla *et al*., 2024). The cattle tick harvours microbial agents like *Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp. *Ricketsia* spp. and *Theileria* spp., posing a significant threat to cattle (Piloto-Sardiñas *et al*., 2023). The main method of tick control includes the application of chemicals; however, the indiscriminate use of these products results in tick resistance, decreasing their effectiveness (Wężyk *et al*., 2025). In addition, the use of chemicals poses a risk to ecosystems and public health (Makwarela *et al*., 2025). To address problems of resistance to chemical methods, there are environmental friend alternatives for tick control, these include plant extracs (Bisht et al., 2025) and entomopathogenic fungi (da Costa-Angelo *et al*., 2025).

The fungi of the genus *Beauveria* spp. (Alali *et al*., 2019) and *Metarhizium* spp. (Beys-da-Silva, 2020) are applied in the field to

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control some arthropods, including *R. microplus* ticks. It is known that these fungi are safe for humans, non-target organisms, and the environment (Ahirwar & Singh 2023, Gutiérrez-Cárdenas *et al*., 2024, Fathy *et al*., 2025). Undoubtedly, the use of acaricides worldwide continues to be used, however, the misuse of chemical products for tick control has led to resistance to them (Obaid *et al*., 2022, De Rouck *et al*., 2023, Cossio-Bayugar *et al*., 2024, Rojas-Cabeza *et al*., 2025). As an alternative to the chemical control, various formulations have been investigated over the years and commercial production has increased considerably (Rajput *et al*., 2024). This article reports the *in vitro* mortality of partially engorged *R. microplus* females, using different concentrations of a *Beauveria bassiana* and *Metarhizium anisopliae* commercial product by immersion test.

MATERIALS AND METHODS

Tick collection and Experiment

The ticks were collected in a farm of El Pitahayo community, located in Cuajinicuilapa, Guerrero, México. The production unit has small livestock, dedicated to the breeding, milking and sale of bovines. The experiment was carried out in the Multidisciplinary Laboratory of the Faculty of Veterinary Medicine and Zootechnics No. 2 of the Autonomous University of Guerrero, located between 16°28'34" N and 98°25'46" W, at an altitude of 46 masl. Once in the laboratory, the ticks were identified by using appropriate keys as *R. microplus* females (Salinas-Estrella *et al*., 2023). The specimens were maintained in a room under 30-35 C, 50-60% of relative humidity, and exposed to a 12-h photoperiod of sunlight through a window.

Product description (Aniquim®)

The product is a commercial biopesticide in liquid formulation with spores of *Metarhizium anisopliae* and *Beauveria bassiana*, at a concentration of $0.5x10^8$ CFU/mL, for each entomopathogenic fungi.

Treatments

In brief, 2.5, 5.0, 7.5, and 10.0 mL of product were diluted in 7.5, 5.0, 2.5, and 0.0 mL, of distilled water dilutions for a final volume of 10 mL. For each treatment, the quantity of product was measured with sterile plastic syringes (Plastipak® - 10 mL) and placed in plastic labeled disposable cups (Plastic World® - 100 mL). The groups were assigned as follows: T1 = 25% product (P) - 75% distilled water (Dw), T2 = 50% P - 50% Dw, T3 = 75% P - 25 % ml Dw, T4 = 100% P, and a control group (Ct) – 100% Dw.

Tick selection

The ticks were selected and classified according to their physical appearance, size, and mobility. All ticks that did not show signs of mobility or were weak, that their size was very different or that showed any physical damage that could modify the results, were discarded. In total, 50 ticks were selected, and 10 individuals were assigned for each treatment group: T1-T4 and Ct. The number of ticks assigned for each treatment was done in accordance with sample size stablished in Martínez-García *et al*. (2023).

Experimental model

Each tick group were placed inside the containers with its respective dilution. Every group were left submerged for 10 minutes (Broglio-Micheletti *et al*., 2012), and once completing the time, were extracted from the container. The excess of the solution was removed with blotting paper, and each tick was returned to their Petri dish. The observation of the specimens was carried at 24-hour intervals (Bravo *et al*., 2008).

Mortality measurement and Data analysis

The daily mortality rate began to be evaluated from 72 hours after treatment (Broglio-Micheletti *et al*., 2012). To observe the treatment effect, the ticks were placed on sheets marked with red circles, expecting to observe mobility in a period of 10 minutes (Giannelli *et al*., 2012). The specimens were considered alive when showed movement in at least one of its legs (Guerrero & Guerrero, 2017). To consider the study viable, the mortality of the control group should not exceed 10% during the observation time (Davey *et al*., 1980). The mortality rate was evaluated as in Martínez-García *et al*. (2023).

RESULTS AND DISCUSSION

In this study, we report the mortality of partially engorged *R. microplus* females, using different concentrations of a *Beauveria bassiana* and *Metarhizium anisopliae* commercial product by *in vitro* immersion test. During the experiment, the different treatments showed an ixodicide effect. The daily mortality attributed to treatment was recorded from the third day after immersion at all concentrations and are showed in the Figure 1. In the control group, mortality was observed from 264 hours, with two specimens, which was maintained until the end of the evaluation. In the treated groups, mortality began at 96 h for T4, at 120 h for T2, at 144 h for T3, and at 192 h for T1. The T4 group showed the highest mortality at 264 h (80%), while the T1 and T3 groups showed their highest mortality at 336 h (60%), finally, the T2 group at 360 h (50%). After 15 days of treatment, all remaining live specimens were sacrificed and discarded.

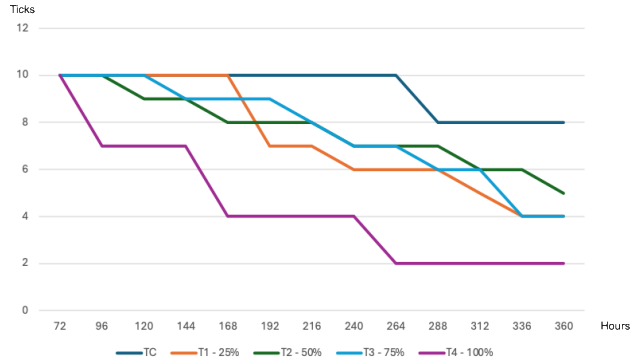

Figure 1. Number of dead specimens with different concentrations of *B. bassiana* and *M. anisopliae* tests.

The genus *Beauveria* (Ascomycota: Hypocreales) is found in different regions around the world and is currently used as an alternative for the biological control of various arthropods, where *B. bassiana* is the most used (Manfrino *et al*., 2024). *B. bassiana* penetrates the host through mechanical force and the production of enzymes that alter the proteins, chitin, and lipids of the host cuticle. Its highly chitinolytic activity is particularly associated with lipase and protease activity, as well as the interaction of hydrolytic enzymes, which confers high activity (Golzan *et al*., 2023). In their study, Sun *et al*. (2013) tested the susceptibility of engorged female of *R. microplus* to three isolates of *B. bassiana* under laboratory conditions; all treatments showed lethal activity at concentrations of 10^7, 10^8 and 10^9 conidia/ml, between 11 and 14 days. While Martínez-García *et al*. (2023) report that mortality from *B. bassiana* at concentrations of 10^6, 10^7, 10^8, and 10^9 on engorged female *R. microplus* began on the third day and ended on the 23rd day post-treatment. In our study, mortality was observed from the fourth day until 15 days post-treatment; these data are consistent with those observed by the mentioned authors.

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The genus *Metarhizium* (Hypocreales: Clavicipitaceae) has a worldwide distribution, contains ascomycete fungi, and is known for its properties for the biological control of arthropods, such is the case of *M. anisopliae* (Brunner-Mendoza *et al*., 2018). *M. anisopliae* begins infection by attaching conidia to the host's cuticle; once attached, the conidia produce a germ tube and release lipases, chitinases, and proteases to rupture and penetrate the cuticle. In the hemocoel, the fungus produces hyphal bodies or blastospores, which release a mixture of extracellular metabolites that interfere with the host's innate immune system. Finally, the hyphae colonize the host, absorbing nutrients which, once depleted, cause the fungi to emerge from the body to produce conidia (Bitencourt, *et al*., 2023). In the investigation by Fernández-Salas *et al.* (2017), the mortality of ticks *R. microplus* subjected to the treatments was observed from four days onwards, ending on day 20, at a dose of 1×108 conidia/mL. For their part, Frazzon *et al*. (2000) observed that the treatments produced mortality from day five until day 14 after treatment on ticks immersed in 10^8 spores/ml of conidial suspensions. Our results are similar to the time ranges in which tick mortality is reported in the studies of the aforementioned authors.

In our study, we evaluated the mortality of the combination of the fungi *B. bassiana* and *M. anisopliae*. There is also a study using a different commercial product than the one used in our trial. The authors report that immersion tests of adult female *R. microplus* subjected to six different concentrations of fungal solutions showed mortality of up to 85%; however, they do not specify the days on which mortality began, the last of which was recorded after 15 days (Romero-Salas *et al*., 2022).

All treatments caused mortality over the ticks subjected to the experiment, even in the lowest concentration solution. In this experiment the employed product showed to be an *in vitro* viable alternative for the mortality of partially engorged *R. microplus* female ticks. The differences in the studies indicate that the mortality rate is proportional to the concentration of conidia. Although all the treatments showed ixodicidal activity, studies are necessary to evaluate the efficacy of these dilutions over fully engorged ticks and their biological parameters, as well as ovicidal and larvicidal capacity.

CONFLICT OF INTEREST

The Author declares no conflict of interest.

REFERENCES

1. Ahirwar, N. K. & Singh, R. (2023). Assessing the safety and efficiency of entomopathogenic fungi as bioinsecticides: A brief review. Journal of Earth & Environmental Waste Management, 1(1): 1-6.
2. Alali, S., Mereghetti, V., Faoro, F., Bocchi, S., Al Azmeh, F. & Montagna, M. (2019). Thermotolerant isolates of *Beauveria bassiana* as potential control agent of insect pest in subtropical climates. PLoS ONE, 14(2): e0211457.
3. Beys-da-Silva, W. O., Rosa, R. L., Berger, M., Coutinho-Rodrigues, C. J. B., Vainstein, M. H., Schrank, A., Bittencourt, Vâ. R. E. P., & Santi, L. (2020). Updating the application of *Metarhizium anisopliae* to control cattle tick *Rhipicephalus microplus* (Acari: Ixodidae). Experimental Parasitology, 208: 107812.
4. Bisht, N., Fular, A., Ghosh, S. & Nanyiti, S. (2025). Systematic review and meta-analysis of acaricidal properties of plant derived products against Ixodid ticks population. Discover Applied Sciences, 7(189), 2025.
5. Bitencourt, R.d.O.B., Santos-Mallet, J.R.d., Lowenberger, C., Ventura, A., Gôlo, P.S., Bittencourt, V.R.E.P., & Angelo, I.d.C. (2023). A novel model of pathogenesis of *Metarhizium anisopliae* propagules through the midguts of *Aedes aegypti* larvae. Insects, 14(4): 328.
6. Bravo, M. J., Coronado, A., & Henríquez, H. (2008). *In vitro* efficacy of amitraz on *Boophilus microplus* populations from dairy farms in Lara state, Venezuela. Zootecnia Tropical, 26(1), 0798-7269.
7. Broglio-Micheletti, S. M. F., de Souza, L. A., Valente, E. C. N., de Araújo, M. J. C., da Silva-Dias, N. & Gómez-Torres, M. L. (2012). Evaluation of entomopathogenic fungi as biological control agents *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini, 1887) (Acari: Ixodidae). Idesia (Arica), 30(1), 93-99.
8. Brunner-Mendoza, C., Reyes-Montes, MR., Moonjely, S., Bidochka, MJ. & Toriello, C. (2018): A review on the genus *Metarhizium* as an entomopathogenic microbial biocontrol agent with emphasis on its use and utility in Mexico, Biocontrol Science and Technology; 29(2): 1-20.
9. Cossio-Bayugar, R., Martinez-Ibañez, F., Aguilar-Diaz, H., & Miranda-Miranda, E. (2024). Relationship between acaricide resistance and acetylcholinesterase gene polymorphisms in the cattle tick *Rhipicephalus microplus*. Parasite (Paris, France). 31: 3.
10. da Costa Angelo, I., Ramirez, J. L., Perez de Leon, A. & Golo, P. (2025). Current perspectives on the use of entomopathogenic fungi for pest control. Frontiers Fungal Biology. 6: 2025.
11. Davey, R. B., Garza, J., Thompson, G. D., & Drummond, O. (1980) Ovipositional biology of the cattle tick, Boophilus annulatus (acari: ixodidae), in the laboratory. Journal of Medical Entomology; 17(3): 287-289.
12. De Rouck, S., İnak, E., Dermauw, W., & Van Leeuwen, T. (2023). A review of the molecular mechanisms of acaricide resistance in mites and ticks. Insect Biochemistry and Molecular Biology. 159: 103981.
13. Fathy, Z., Muhammad, J. & Ali, S. S. (2025). The pathology of native entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae), on biological processes and ovarian structure of the African migratory locust, *Locusta migratoria migratorioides* (Orthoptera: Acrididae). Egyptian Journal of Biological Pest Control. 35: 6.
14. Fernández-Salas, A., Alonso-Díaz, M. A., Alonso-Morales, R. A., Lezama-Gutiérrez, R., Rodríguez-Rodríguez, J. C., & Cervantes-Chávez, J. A. (2017). Acaricidal activity of *Metarhizium anisopliae* isolated from paddocks in the Mexican tropics against two populations of the cattle tick *Rhipicephalus microplus*. Medical and Veterinary Entomology, 31(1): 36-43.
15. Frazzon, A. P., da Silva Vaz Junior, I., Masuda, A., Schrank, A., & Vainstein, M. H. (2000). *In vitro* assessment of *Metarhizium anisopliae* isolates to control the cattle tick *Boophilus microplus*. Veterinary Parasitology, 94(1-2): 117-125.

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16. Giannelli, A., Dantas-Torres, F. & Otranto, D. (2012). Underwater survival of *Rhipicephalus sanguineus* (Acari: Ixodidae). Experimental & Applied Acarology, 57(2), 171-178.
17. Golzan, S.R., Talaei-Hassanloui, R., Homayoonzadeh, M. & Safavi, S.A. Role of cuticle-degrading enzymes of *Beauveria bassiana* and Metarhizium anisopliae in virulence on Plodia interpunctella (Lepidoptera, Pyralidae) larvae. Journal of Asia-Pacific Entomology. 26:102038 (2023).
18. Guerrero, B. & Guerrero, P. E. (2017) Evaluación del extracto de Neem (*Azadirachta indica*), para controlar garrapatas (*Boophilus microplus*), en ganado bovino. Visión Antataura. 1(1): 16-27.
19. Gutiérrez-Cárdenas, O. G., Cortez Madrigal, H. & Garzón, A. (2024). Use and impact of endophytic entomopathogenic fungi: Their potential in the context of agricultural sustainability. Biotecnia, 26: e1986.
20. 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.
21. Makwarela, T. G., Seoraj-Pillai, N., & Nangammbi, T. C. (2025). Tick control strategies: Critical insights into chemical, biological, physical, and integrated approaches for effective hard tick management. Veterinary Sciences, 12(2): 114.
22. Manfrino, R., Gutierrez, A., Ben Gharsa, H., Schuster, C., López Lastra, C., & Leclerque, A. (2024). Molecular taxonomic characterization and infra-specific diversity of entomopathogenic *Beauveria bassiana* fungi from Argentina. Fungal Biology, 128(3): 1800-1805.
23. Martínez-García, J., Abad-Zavaleta, J., García-Gómez, M. J., & Núñez-Gaona, O. (2023). *In vitro* evaluation of the acaricidal potential of *Beauveria bassiana* DS3.17 on the common tick (*Rhipicephalus microplus*) in Oaxaca, Mexico. Revista Mexicana de Ciencias Pecuarias, 14(3): 644-657.
24. Obaid, M. K., Islam, N., Alouffi, A., Khan, A. Z., da Silva, Vaz. I. Jr., Tanaka, T. & Ali, A. (2022). Acaricides resistance in ticks: Selection, diagnosis, mechanisms, and mitigation. Frontiers in Cellular and Infection Microbiology, 12: 941831.
25. Piloto-Sardiñas, E., Foucault-Simonin, A., Wu-Chuang, A., Mateos-Hernández, L., Marrero-Perera, R., Abuin-Denis, L., Roblejo-Arias, L., Díaz-Corona, C., Zając, Z., Kulisz, J., Woźniak, A., Moutailler, S., Corona-González, B., & Cabezas-Cruz, A. (2023). Dynamics of infections in cattle and *Rhipicephalus microplus*: A preliminary study. Pathogens, 12(8): 998.
26. Rajput, M., Sajid, M. S., Rajput, N. A., George, D. R., Usman, M., Zeeshan, M., Iqbal, O., Bhutto, B., Atiq, M., Rizwan, H. M., Daniel, I. K., & Sparagano, O. A. (2024). Entomopathogenic fungi as alternatives to chemical acaricides: Challenges, opportunities and prospects for sustainable tick control. Insects, 15(12): 1017.
27. Rojas-Cabeza, J. F., Moreno-Cordova, E. N., Ayala-Zavala, J. F., Ochoa-Teran, A., Sonenshine, D. E., Valenzuela, J. G., & Sotelo-Mundo, R. R. (2025). A review of acaricides and their resistance mechanisms in hard ticks and control alternatives with synergistic agents. Acta Tropica, 261: 107519.
28. Salinas-Estrella, E., Bautista-Garfias, C. R., Martínez-Ibañez, F., García-Ortíz, M. A., Rodríguez-Camarillo, S. D. & Cossío-Bayúgar, R. (2023). Chapter 2 *Rhipicephalus microplus*: Tick Classification, Morphological Identification and Life Cycle. In: Cossio-Bayugar, R., Miranda-Miranda, E. & Kumar, S. (Ed). A Laboratory Manual on *Rhipicephalus microplus* (pp. 13-29). Cambridge Scholars Publishing. UK.
29. Sun, M., Ren, Q., Guan, G., Li, Y., Han, X., Ma, C., Yin, H., & Luo, J. (2013). Effectiveness of *Beauveria bassiana* sensu lato strains for biological control against *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) in China. Parasitology International, 62(5): 412-415.
30. Wężyk, D., Bajer, A. & Dwużnik-Szarek, D. (2025). How to get rid of ticks – a mini-review on tick control strategies in parks, gardens, and other human related environments. Annals of Agricultural and Environmental Medicine, 32(1): 1-8.
31. Nejash, A. (2016). Review of important cattle tick and its control in Ethiopia. Open Access Library Journal, 3: 1-11.
32. Ostfeld, R. S. Price, A., Hornbostel, V. L., Benjamin, M. A. & Keesing, F. (2006). Controlling ticks and tick-borne zoonoses with biological and chemical agents. BioScience, 56(5), 383-394.
33. Rodriguez-Vivas, R. I., Jonsson, N. N. & Bhushan, C. (2018). Strategies for the control of *Rhipicephalus microplus* ticks in a world of conventional acaricide and macrocyclic lactone resistance. Parasitology Research, 117(1), 3-29.
34. Romero-Salas, D., Bravo-Ramos, J. L., Sanchez-Montes, D. S., Cardenas-Amaya, C., Gamboa-Prieto, J., Cruz-Romero, A., & Olivares-Muñoz, A. (2022). Acaricidal activity of combined commercial strains of entomopathogenic fungi against *Amblyomma mixtum* and *Rhipicephalus microplus* ticks. Tropical and Subtropical Agroecosystems, 25(3): 113.
35. Showler, A. T. & Saelao, P. (2022). Integrative alternative tactics for ixodid control. Insects, 13: 302.
36. Uspensky, I. V. (2017). Blood-sucking ticks (Acarina, Ixodoidea) as an essential component of the urban environment. Entomological Review, 97(7): 941-969.
37. Vudriko, P., Okwee-Acai, J., Byaruhanga, J., Tayebwa, D. S., Okech, S. G., Tweyongyere, R., Wampande, E. M., Okurut, A., Mugabi, K., Muhindo, J. B., Nakavuma, J. L., Umemiya-Shirafuji, R., Xuan, X. & Suzuki, H. (2018). Chemical tick control practices in southwestern and northwestern Uganda. Ticks and Tick-borne Diseases, 9(4): 945-955.

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