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Control biológico de Rhicephalus (Boophilus) microplus con hongos entomopatógenos

Biological Control of Rhicephalus (Boophilus) microplus with Entomopathogenic Fungi

Controle biológico de Rhicephalus (Boophilus) microplus com fungos entomopatogênicos

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Resumen

En México el problema principal de la ganadería bovina, en lo particular en el trópico, es controlar las garrapatas *Rhicephalus (Boophilus) microplus*, ya que el uso inadecuado de los tratamientos químicos ha creado resistencia en esos parásitos. El control biológico en garrapatas es un concepto nuevo y ha sido una medida efectiva, que ha permitido mantener estas dentro de parámetros económicos y zoosanitarios aceptables. Una de las posibilidades reales en este campo para controlar garrapatas *Rhicephalus (Boophilus) microplus* de importancia pecuaria es el uso de hongos entomopatógenos como el *Metharrizium anisopliae* y *Beauveria bassiana*. La finalidad del presente estudio fue observar la capacidad patógena de dos cepas de *Metarhizium anisopliae* a dosis de $1X10^8$ conidias/ml y $1.3X10^{12}$ conidias/ml, y de una cepa de *Beauveria bassiana* a una dosis de $1.3X10^{12}$ conidias/ml en unidades de producción en bovinos de doble propósito, en los municipios de Emiliano Zapata

Tabasco y la Cuenca Lechera de Catazajá, Chiapas, México; directamente en campo. Se presentó una respuesta binomial negativa de mayor porcentaje de patogenicidad en las dosis de 1.3X10^{12} conidias/ml de *Beauveria bassiana* con 76.66% a los 37.3 días de haber sido inoculada la garrapata, en contraste con las dos cepas de *Metarhizium anisopliae*, que mostraron una patogenicidad de 47.71% a los 10 días con la dosis 1X10^{8} conidias/ml y de 37.75% a los 44.5 días con la dosis 1.3X10^{12} conidias/ml. Se presentó interacción entre tratamiento y tiempo. Por tanto, se concluye que el uso de los hongos entomopatógenos son una alternativa para el control de garrapatas adultas en el sureste de México.

Palabras clave: Metarhizium anisopliae, Beauveria bassiana, garrapata, bovino.

Abstract

In Mexico, the main problem of bovine cattle breeding, particularly in the tropics, is to control the ticks Rhicephalus (Boophilus) microplus, since inappropriate use of chemical treatments has created resistance in these parasites. Biological control in ticks is a new concept and has been an effective measure, which has allowed to keep these within acceptable economic and animal sanitary parameters. One of the real possibilities in this field to control ticks Rhicephalus (Boophilus) microplus of animal importance is the use of entomopathogenic fungi such as Metharrizium anisopliae and Beauveria bassiana. The aim of the present study was to observe the pathogenic capacity of two strains of Metarhizium anisopliae at doses of 1X108 conidia/ml and 1.3X1012 conidia/ml and of a strain of Beauveria bassiana at a dose of 1.3X1012 conidia/ml in production units In dual purpose cattle, in the municipalities of Emiliano Zapata Tabasco and the Cuenca Lechera de Catazajá, Chiapas, Mexico; Directly in the field. A negative binomial response with a higher percentage of pathogenicity was observed at the doses of 1.3X1012 conidia/ml of Beauveria bassiana with 76.66% at 37.3 days after the tick was inoculated, in contrast to the two strains of Metarhizium anisopliae, which showed a Patogenicity of 47.71% at 10 days with the dose 1X108 conidia/ml and 37.75% at 44.5 days with the dose 1.3X1012 conidia/ml. Interaction between treatment and time was presented. Therefore, it is concluded that the use of entomopathogenic fungi are an alternative for the control of adult ticks in southeastern Mexico.

Key words: Metarhizium anisopliae, Beauveria bassiana, tick, bovine.

Resumo

No México o principal problema de gado, em especial, nos trópicos, é controlar Rhicephalus (Boophilus) microplus carrapatos, como o uso indevido de tratamentos químicos criou resistência em estes parasitas. O controle biológico nos carrapatos é um conceito novo e tem sido uma medida efetiva, que permitiu mantê-los dentro de parâmetros econômicos e de saúde animal aceitáveis. Uma das possibilidades reais neste campo para Rhicephalus carrapatos (Boophilus) microplus importância gado é o uso de fungos entomopatogicos tais como Beauveria bassiana e Metharrizium anisopliae. O objectivo deste estudo foi observar a patogenicidade de duas doses Metarhizium anisopliae de 1x108 / ml e 1.3X1012 conídios / ml, e uma estirpe de Beauveria bassiana a uma dose de 1.3X1012 conídios / ml em unidades de produção gado de duplo propósito, nos municípios de Emiliano Zapata Tabasco e Catazajá Cuenca Lechera, Chiapas, México; diretamente no campo. Mostrou uma resposta binomial negativo maior percentagem de patogenicidade em doses 1.3X1012 conídios / ml de Beauveria bassiana com 76.66% para 37,3 dias de carrapato inoculado, em contraste com as duas estirpes de Metarhizium anisopliae, que eles mostraram patogenicidade 47,71% em 10 dias com a dose de 1x108 conídios / ml e 37,75% para 44,5 dias com uma dose 1.3X1012 conídios / ml. Interação entre tratamento e tempo foi apresentada. Portanto, conclui-se que o uso de fungos entomopatogênicos é uma alternativa para o controle de carrapatos adultos no sudeste do México.

Palavras-chave: Metarhizium anisopliae, Beauveria bassiana, carrapato, bovino.

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Introduction

Ticks significantly affect extensive livestock farming and even endanger the health of humans either directly or through inoculation of pathogenic organisms (Alvarez, 2007). There are pathologies that the ticks can transmit to the man even if he is not his habitual host. The International Epidemiological Alert Bulletin mentions some of the following: Lyme disease, Recurrent fever, Rickettsial fevers, Ehrlichiosis Anaplasmosis, Tularemia Babesiosis, Virus encephalitis, Colorado fever, among others (Quiroz, 2007). In the tropics there are several types of ticks; in Mexico 77 species of ticks have been identified that affect cattle and man (Rodríguez-Vivas et al., 2006); the geographic distribution of tick species in Mexico depends on environmental factors, eg, relative humidity, temperature and vegetation. Other factors are the altitude, presence and abundance of hosts and control or eradication practices that man exerts on tick populations (Rodríguez-Vivas et al., 2006; Estrada-Peña et al., 2006).

The following species of ticks are identified in national cattle breeding: Boophilus microplus, B. anulatus, Amblyomma cajennense, A. imitator. A. maculatum, A. triste, A. americanum and Anocentor nitens; However, the species of major importance for cattle in Mexico are B. microplus and A. cajennense (Rodríguez-Vivas et al., 2006). The range of the Rhipicephalus (Boophilus) microplus comprises tropical, temperate and arid areas; together it is considered to cover 1 043 772 km2, which represents 53.0% of the national territory. The population dynamics of Rhipicephalus (Boophilus) microplus in the states of Chiapas and Tabasco occur throughout the year, but in winter it is estimated that it can live up to 51 days despite climatic conditions (Cortes et al., 2010). The tick Rhipicephalus (Boophilus) microplus is the ectoparasite that produces greater economic losses in the Mexican tropic and subtropics, due to the high costs associated with its control and the diseases that it transmits (Rodríguez-Vivas *et al.*, 2005).

The means for tick control are various ixodicides, such as arsenicals, organochlorines, organophosphates, carbamates, formamides, synthetic pyrethroids (flumethrin), acrocyclic lactones (eprinomectin), phenylpyrazoles (fipronil), as well as some growth regulators such as analogues of the juvenile hormone (methoprene and fenoxycarb) and inhibitors of chitin synthesis, such as fluazuron, diflubenzuron, lufenuron and cyromazine (Cordero et al., 1999 and Cuore et al., 2008 and Rodríguez et al., 2010) . The ixodicides have been used with good results in the control of ticks; however, their continuous and irrational use has generated resistant strains, such as in the Catazajá (CLC), Chiapas and Tabasco (AM) in the municipality of Emiliano Zapata, Tabasco and in the states of Chiapas, Yucatan and Quintana Roo, since 88% of herds are resistant to pyrethroids (PS) and (AO) (Fuentes, 2011, Araque, A et al., 2014), so that producers have been increasing the number of applications of chemical products (SENASICA) in periods ranging from 8 to 21 days, that high production costs are increasing (Monroy et al., 2016), the demand for food free of chemical residues and care for the environment have led to the need to search for non-chemical alternatives for tick control, based on the use of fungi and ntomopathogens that have been shown to be effective for the control of several genera of these parasites, including Amblyomma and Rhipicephalus. The species of entomopathogenic fungi that have demonstrated efficacy against ticks are Beauveria bassiana, Metarhizium anisopliae, Isaria farinosa (= Paecilomyces farinosus) and Lecanicillium lecanii (= Verticillium lecanii), with B. bassiana and M. anisopliae being the most studied species and most (Fernandes and Bittencourt, 2008, Arguedas et al., 2008, López et al., 2009, Fernández et al., 2005, 2010, Ojeda-Chi et al., 2011, Motta et al., 2011, Bautista (1999), and the results obtained in the present study were similar to those reported in the present study. , Valverde et al., 2015, Tinajero et al., 2016). The objective of the present study was to evaluate the pathogenic capacity of M. anisopliae and B. bassiana entomopathogenic fungi in adult Rhodocopalus (Boophilus) microplus ticks in dual purpose bovine herds in the Rio de Tabasco, and the Dairy Basin of Catazajá, Chiapas, Mexico. The entomopathogenic fungi gradually reduce the infestation of the tick Rhicephalus (Boophilus) microplus in the tropics and reduce the use of agrochemicals used by producers to control them.

Materials and methods

Characterization of Dual Purpose Cattle Production Units

For the selection of the experimental units, a sample consisting of 62 cooperating active cattlemen from the communities of Agua Fría, Punta Arena, Chiapas and Emiliano Zapata in Tabasco was previously taken. This made it possible to capture the variability of conditions and characteristics of bovine milk production systems. In order to classify and analyze the bovine production systems, information was obtained through the semi-structured informal interview technique (Gillham, 2005), and direct observations were made in the field. A questionnaire was generated that generated a series of indicators that were grouped as follows: Size of the herd (UA), Birth rate (%), Infant mortality rate (%), Adult mortality rate (%), Level of use of mechanical equipment (%), Level of use of mechanized equipment (%), Infrastructure characteristics (%), Animal load (UA / ha), Disease control degree , Number of cows per stallion (n), Characteristic of accommodation and facilities of the production unit (%), Means of work (%). The mean values of the indicators and the variables were examined statistically in the program Statistical Package for Social System (SPSS 2012), version 22.0.

Field trials

The trial was carried out in four production units in dual purpose cattle from the Catazajá Dairy Basin, located in the north of the state of Chiapas, Mexico; in the parallels 17 ° 12 'and 17 ° 57' north latitude, meridians 91 ° 46 'and 92 ° 12' west latitude; altitude between 0 and 10 msnm, adjacent to the north, east and west with the state of Tabasco and the municipality of Palenque, and to the south with the municipality of Palenque (INEGI, 2010), and a production unit in double cattle purpose in the municipality of Emiliano Zapata, Tabasco.

Five randomly selected cattle, adults with an average weight of 450 kg live, were selected under free grazing conditions with pasture rotation, fed with natural grass (Paspalum notatum). It is important to mention that four weeks before the study AmitrazMR was applied by spraying in doses of 2 ml L-1 of water. To carry out the applications in the field tests, two strains of the fungus M. anisopliae and one strain of B. bassiana were previously selected. The laboratory results showed a greater pathogenicity in adult ticks.

Next, counts of adult ticks were made on cattle prior to application. The ticks of more than 4 mm in length, present in 20 cm2 of skin in six predetermined areas (table of necks on both sides, the two English or crotch and both armpits of the previous members) were taken as criterion. (Bazán et al., 2008; Balladares et al., 2014), which consisted of an acetate sheet with a rectangular groove 2 cm wide and 10 cm long (20 cm2).

The treatments consisted of two strains of Metarhizium anisopliae evaluating a native strain MM01 at a concentration of $1X10^8$ conidia / ml, provided by the Laboratory of Biotechnology of the Maya School of Agricultural Studies of the Autonomous University of Chiapas, a commercial strain at a concentration of 1.3X1012 conidia / ml and a strain of Beauveria bassiana at the concentration of 1.3x1012 conidia / ml, provided by the laboratory Tiemelonlá Nich Klum of Palenque, Chiapas; to those strains evaluated at the time of application to cattle were added an adherent consisting of 0.03% TweenMR 20, also a chemical treatment (Amitraz ™) was evaluated at the commercial dose recommended by the laboratory of 2 ml L-1 of water and another treatment that served as control using only water of 2.5 liters per animal, the same amount as in the previous groups.

Before starting with the application, the count of live ticks was recorded and then the treatments were applied. Each group of cattle received three treatments spaced with 15 days, the application was made by spraying baths with a manual 20-liter backpack pump, during the period from October 2013 to November 2016, only during the rainy season due to relative humidity required by the fungus to exert its fungicidal function on plethora ticks (Bautista et al., 2016).

After each application the tick count was monitored, then transferred to the laboratory with the support of a humid chamber, consisting of sterile disposable plastic Petri boxes of 100x15 mm, with sterile filter paper moistened with sterile distilled water at the bottom of the box to retain moisture from 80% to 85%. They were sealed with pre-sterilized parafilm and labeled with the treatment number and identification number of the bovine (Bautista et al., 2016, Pirali-Kheirabadi et al., 2016). In the laboratory the degree of pathogenicity of the fungus in adult live ticks was determined.

Variables

• Pathogenicity of the fungus in ticks. This variable was determined in the Biotechnology Laboratory of the Maya School of Agricultural Studies of the Autonomous University of Chiapas.

• Number of adult live ticks in 20 cm^2 of skin. This variable was determined in the Dual Purpose Cattle Production Units.

Experimental design and data analysis

A completely randomized block design was used, with five treatments and five replicates; each treatment was evaluated in a separate Double Purpose Cattle Production Unit, with the following model:

$$Y_{ij} = \mu + Ø_i + Ø_i(\beta_j) + {}_{Tk} + Ø_{Tijk} + \Sigma_{ijk}$$

Where:

Y_{ij} = response variables.

μ = effect of the population mean.

$Ø_i$ = effect of the incubation period of the fungus on the tick in its parasitic phase.

$Ø_i(\beta_j)$ = nested effect of incubation periods within replicates.

${}_{Tk}$ = effect of the evaluated strains.

$Ø_{Tijk}$ = effect of the interaction between the incubation period and the strains.

Σ_{ijk} = experimental error associated with experimental units.

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Results

Characterization of Dual Purpose Cattle Production Units

Production units in dual-purpose cattle were characterized by having producers at an age of 52 years and a grade of 6.53 years; its system of cattle production has a dual purpose: the production of milk for family consumption and local and regional marketing; 40% destine its production to the commercialization at local level, supplying to small transformers, who add value by means of the transformation to cheese, distribution that can become regional; the remaining 60% of the producers directly sell to the Nestlé transnational company, it has an average of 16.95 hectares per producer and implements its production system through extensive grazing with paddock rotation, so they depend to a great extent on the fodder that there is to feed her herd. Most of the hectares are used for livestock and agriculture, and very few are used for agriculture. The first is the one with the largest area: 16.95 ha, the second occupies 1.26 ha and the third 0.03 ha. Guasimo (Guazuma ulmifolia) with 6.45%, the grass most predominant is the natural Grass (Paspalum notatum) with 35.8%, although they also have other species, for example, the African star (Cynodon nlemfuensis), the insurgent (Brachiaria brizantha) and the signal grass (Brachiaria decumbens); the size of the livestock holding is 43.4 in terms of animal unit (UA), the predominant bovine breed is the Cebu / Swiss cross with 64.05%, and the animal load expressed in animal unit per hectare is considered high with 2.99 AU / ha . In addition, 61.3% of the producers identify their meadows as moderately padded and 17.4% mention that they are in conditions of overgrazing; the excessive number of animal units per hectare has resulted in the income of pastures, an activity that 32% of the producers do, with an estimated 3.54 ha each. Another important activity is the management of the cattle herd, specifically with respect to the degree of disease control. 40.3% of the producers depend on external inputs in 68.5%, which indicates that they are acquiring the necessary inputs to carry out their basic production activities; the mortality in hatchlings was not high, since they obtained in this indicator 10.1% taking into account the conditions of the region. Regarding the reproductive area, there was a high number of cows per stallion with 25.1 females; the most favorable calving season is during the months of April-May and the calves are weaned at six months of age with an approximate weight of 120 kg for fattening and stallions are replaced at four and five years. In terms of accommodation and facilities, 53.5% have basic facilities: corrals, cellars, feeders, drinking troughs, and so on. Only 49.9% of producers have basic services such as roads, electricity and piped water. The lack of adequate facilities and infrastructure results in the use of very little machined equipment with 5.72%, in contrast to the level of use of manual equipment with 86%.

Field trials

The results of the evaluation of the entomopathogenic fungi of Metarhizium anisopliae and Beauveria bassiana for the control of the tick Rhicephalus (Boophilus) microplus in adult state, in production units in dual purpose cattle, municipalities of Emiliano Zapata , Tabasco and the Dairy Basin of Catazajá, Chiapas.

Table 1. Evaluación de los hongos entomopatógenos *Metarhizium anisopliae* y *Beauveria bassiana*, para el control de la garrapata *Rhicephalus (Boophilus) microplus* en estado adulto, en Unidades de Producción Pecuaria, en Emiliano Zapata, Tabasco y la Cuenca Lechera de Catazajá, Chiapas, México.

Tratamiento	Localidad	Ubicación de los hatos (GPS)	No. de garrapatas antes de la aplicación	No. de garrapatas después de la aplicación	% de mortalidad	% de patogenicidad
Metarhizium anisopliae **$1X10^8$ conidias/ml**	Emiliano Zapata, Tabasco	17°45'00.2" N y 91°45'21.5" W	5.6	1.2	78.45	47.71
Metarhizium anisopliae **$1.3X10^{12}$ conidias/ml**	Punta Arena, Catazajá, Chiapas	17°45´04.20´ N y 92°03´35.54” W	68.2	12.4	81.82	37.75
de *Beauveria bassiana* **$1.3x10^{12}$ conidias/ml**	Punta Arena, Catazajá, Chiapas	17°74´69.44” N y 92°05´58.33”, W	26.4	8.6	67.42	76.66
Amitraz 2 ml L-1 de agua	Punta Arena, Catazajá, Chiapas	17°45'20.2" N y 92°03'56.1" W	5.8	4.6	20.45	n.a
Testigo sin aplicación	Agua Fría Catazajá, Chiapas	17°44'12.1" N y 92°05'00.9" W	3.4	6.2	n.a	n.a

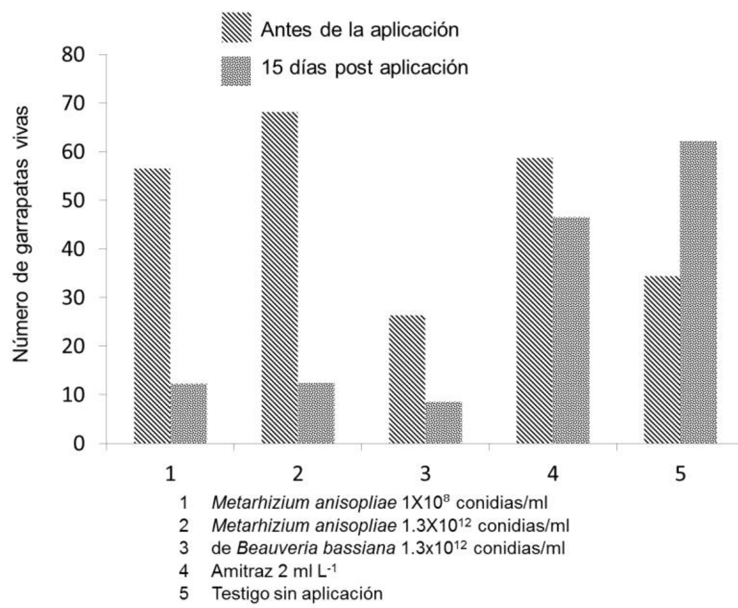
Cuadro 1. n.a, no aplica

Source: elaboración propia.

As can be seen in Table 1, the different treatments evaluated in this study had an effect of up to 76.6% of pathogenicity in adult ticks with Beauveria bassiana strain 1.3X1012 conidia / ml and a range of 37.75% of pathogenicity in ticks with the strain of Metarhizium anisopliae 1.3x1012 conidia / ml.

On the other hand, Figure 1 shows the behavior of each of the treatments performed and the average number of ticks per animal before and after application.

Figure 1. Evaluación de los hongos entomopatógenos de *Metarhizium anisopliae* y *Beauveria bassiana*, para el control de la garrapata *Ricephalus (Boophilus) microplus* en estado adulto, comparando el número de garrapatas vivas antes de la aplicación del tratamiento frente al número de garrapatas vivas a 15 días post aplicación.



Source: elaboración propia.

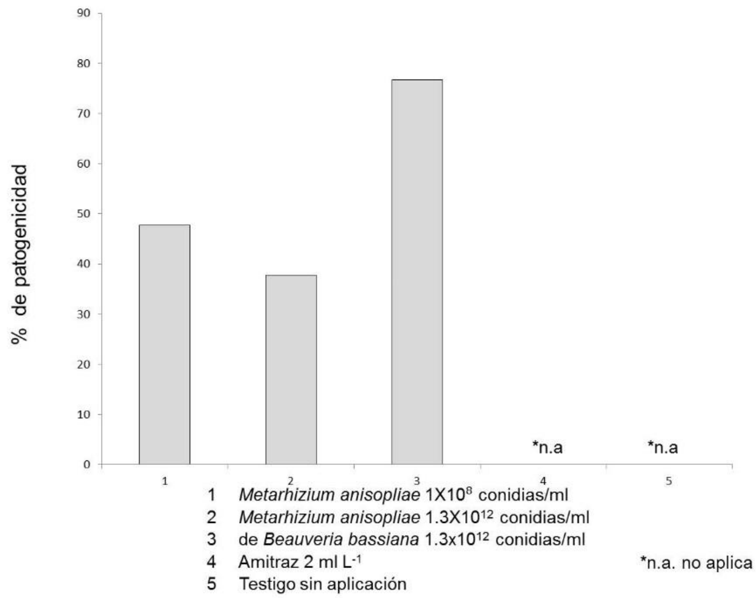
In Figure 1 it can be seen that the number of live ticks before the application of the treatment against the number of live ticks at 15 days of application, with the dose of the native strain MM01 of Metarhizium anisopliae 1X10^8 conidia / ml, average of 56.6 live ticks per animal before application, and 12.2 ticks on average per animal at 15 days of application; in the commercial strain of Metarhizium anisopliae 1.3X1012 conidia / ml an average of 68.2 live ticks per animal was observed before application and 12.4 live ticks on average per animal at 15 days of application; in the commercial strain of Beauveria bassiana 1.3X1012 conidia / ml an average of 26.4 live ticks per animal was observed before application and 8.6 ticks per animal averaged 15 days after application; in the treatment with Amitraz MR at the dose of 2 ml L-1 an average of 58.8 ticks per animal was observed before the application and of 46.6 ticks on average per animal at 15 days of application; in the control treatment without application an average of 34.4 ticks per animal was observed before the application of only water and of 62.2 ticks on average per animal to 15 days of the application of water, in equal amounts as in the previous treatments.

The highest rates of tick mortality per animal occur in the commercial strain of Metarhizium anisopliae 1.3X1012 conidia / ml, then in the native strain of MM01 Metarhizium anisopliae $1X10^8$ conidia / ml and, finally, in the commercial strain of Beauveria bassiana 1.3X1012. In the treatment with AmitrazMR at the dose of 2 ml L-1, it was observed that the treatment of the native strain of Metarhizium anisopliae $1X10^8$ conidia / ml decreased the average number of ticks per animal of 44.4 (78.45%) mortality, treatment with the commercial strain of Metarhizium anisopliae 1.3X1012 conidia / ml decreased the number of average ticks per animal from 55.8 (81.82%) mortality; the treatment with Beauveria bassiana strain 1.3X1012 conidia / ml decreased the number of average ticks per animal of 17.8 (67.42%) and the number of ticks per animal of 12.2 (20.75%) mortality, whereas the control treatment without application increased the number of ticks per animal of 27.8 (80.81%).

Pathogenicity

The pathogenicity effect of the commercial strain of Beauveria bassiana was higher in comparison with the entomopathogenic fungi of the native strains MM01 and commercial of Metarhizium anisopliae.

Figure 2. Patogenicidad de los hongos entomopatógenos de *Metarhizium anisopliae* y *Beauveria bassiana* para el control de la garrapata Ricephalus (Boophilus) microplus, en el municipio de Emiliano Zapata, Tabasco y en la Cuenca Lechera de Catazajá, Chiapas, México.



Source: elaboración propia.

As shown in Figure 2, the treatment of the native strain MM01 of Metarhizium anisopliae 1X108 conidia / ml presented a pathogenicity percentage of 47.71% 10 days after being inoculated in the tick compared to the strain commercial strain of Metarhizium anisopliae 1.3X1012 conidia / ml, which showed 37.75% pathogenicity 44.5 days after being inoculated in the tick, while the commercial strain of Beauveria bassiana 1.3X1012 obtained 76.66% pathogenicity at 37.3 days of being inoculated in the tick.

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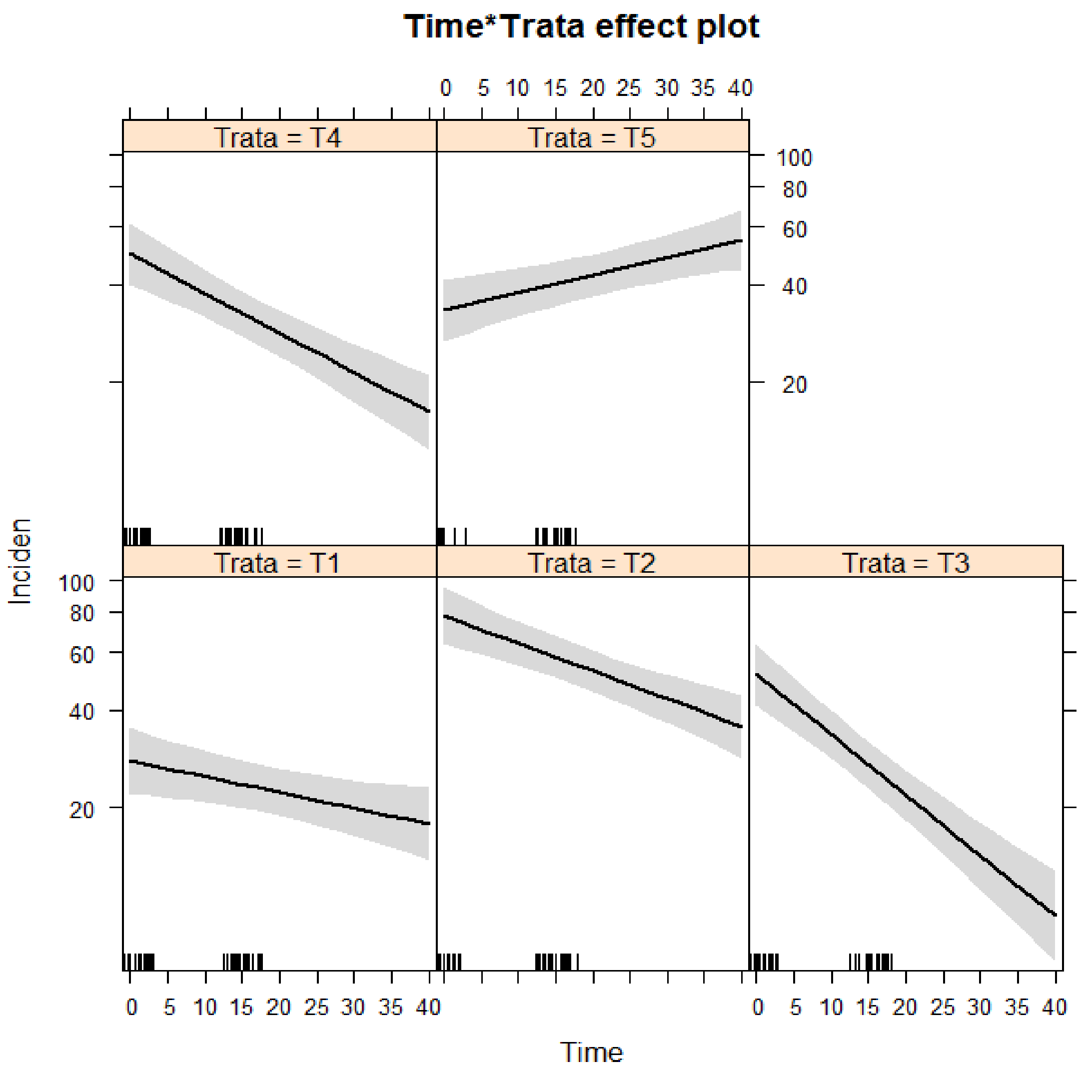
Figure 3. Efecto binomial de los hongos entomopatógenos para el control de la garrapata en estado adulto.



Source: elaboración propia.

Figure 3 shows that the binomial effect was negative and that there is an interaction between treatment and time, that is, the greater the number of days elapsed after the inoculation of the entomopathogenic fungi, the effect of tick mortality decreases in comparison with the control. Therefore, the statistical analysis showed a highly significant difference with the time factor in terms of chi-square data of 66,810 with a probability of 0.001% significance.

Discussion

Entomopathogenic fungi have been shown to have great potential for the biological control of Rhicephalus (Boophilus) microplus (Ojeda-Chi et al., 2011; Fernandes et al., 2012). Under field conditions, pathogenicity may be affected by macro-climatic factors such as temperature, humidity and solar radiation, as well as microclimatic factors such as chemical secretions of the animal's skin, microflora and skin temperature, which contribute to raise the level of infection of the entomopathogenic fungi (germination and penetration) (Fernandes and Bittencourt, 2008, Leemon and Jonsson, 2008, Dantigny and Nanguy, 2009).

Pérez J. (2007) asserts that the mortality caused by Beauveria bassiana (59.19%) in Beauveria bassiana (59.19%) under laboratory conditions is the result of Beauveria bassiana strain 1.3X1012 conidia / ml, with 67.42% under field conditions, repeated applications every 15 days, in hours of low solar radiation (07: 00-08: 00); this percentage of mortality decreased with the passage of the days because there was an interaction between the environment, the ticks of the animal and the fungi, that conditions the effectiveness of the latter. Likewise, Polar et al. (2005) evaluated the effect of solar radiation on body temperature in relation to the survival of the spores of Metarhizium anisopliae, reporting that areas of the body exposed to solar radiation with temperatures higher than 35-40 ° C showed lower efficacy of the fungus, compared to temperatures below 30-35 ° C, in which Metarhizium anisopliae adheres, penetrates and germinates in ticks within the first 24 hours after treatment. Applying the treatments at hours of low solar radiation may have favored the good efficacy of the fungi for the in vivo control of Rhicephalus (Boophilus) microplus. On the other hand, Arguetas et al. (2008) evaluated the efficacy of the entomopathogenic fungus Metharrizium anisopliae in the control of Boophilus microplus (Acari: Ixodidae) and reported the control of Ricephalus (Boophilus) microplus populations when spraying conidia of Metarhizium anisopliae 1X1010 conidia / ml on cattle under conditions of field, which shows a decrease of adult ticks in 79% and Kaaya GP et al. (2011) at a concentration of 1X108 conidia / ml in 83%.

The efficiency of the use of Amitraz® at the dose of 2 ml L-1 in the present study showed a mortality rate of 20.75%, results that do not coincide with those of Arguetas et al. (2008), as the latter reported control of Ricephalus (Boophilus) populations by using Amitraz ™ as a control treatment, which showed a decrease in adult ticks by 59%. Possibly the climatic conditions between the two studies were different.

The strains of Metarhizium anisopliae in native dose of 1X108, the commercial dose of 1.3X1012 and the commercial strain of Beauveria bassiana 1.3X1012 decreased tick infestation in the animals. This control effect of these entomopathogenic fungi on ticks was conserved in treated animals for two weeks after the last application, similar results were observed by Kaaya et al. (1996), for whom the effect of conidia of the fungi of Beauveria bassiana and Metarhizium anisopliae persisted between one and three weeks in the ears of the animals after the application.

Alonso-Díaz et al. (2007), in their evaluation of Metarhizium anisopliae (Hyphomycetes) for the control of Boophilus microplus (Acari: Ixodidae) in natural infestations in the Mexican tropics, reported efficacy of 40 to 91% using the Ma34 strain of Metarhizium anisopliae on bovines infested with adult ticks of Rhicephalus (Boophilus) microplus, which coincides with that reported by this study where the range was 37.33 to 44.5% of pathogenicity. This may have favored the animal due to macro and microclimatic conditions (Edmundo Cabrera, 2013); However, in the pastures where the treated animals grazed, after 20 days of the occupation period they grazed others that were not treated, which presented less infestation of ticks, this verifies the dispersion of the spores mentioned by Bautista et al. (2012).

Polar et al. (2005), in the research on the thermal characteristics of Metarhizium anisopliae isolates important for the development of biological pesticides for the control of ticks, reported efficacies of <50% at concentrations of 1 X 108 conidia / ml in livestock reared under natural infestations, which coincides with the results of this study at the same concentration 1 X 108 conidia / ml of Metarhizium anisopliae, only under free grazing conditions.

Leemon et al. (2008) evaluated the efficacy of Metarhizium anisopliae in laboratory and field conditions on cattle; these authors reported low tick mortality on animals compared to mortality obtained under laboratory conditions, because microclimatic factors of animals negatively affect spores that inoculate on ticks during the spray process. The results obtained by them coincide with what was found in the present study, where the efficacy of the entomopathogenic fungi used did not reach 90-100% as previously happened under laboratory conditions (Ojeda-Chi *et al.*, 2010).

Pulido et al. (2015) evaluated the strain MaF1309® of Metarhizium anisopliae in the biological control of adult ticks of Rhicephalus microplus in Tunja, Colombia, where they reported that ticks treated with the strain MaF1309® of Metarhizium anisopliae at a concentration of 1 X 108 conidia / ml reached 100% mortality at 14 days after treatment, and that 50% of the ticks presented fungus at 10, 18 and 22 days of treatment, which is consistent with the results of this study with the native strain MM01 at the same concentration 1 X 108 conidia / ml, which showed pathogenicity in ticks at 10 days after application, however, does not coincide with the post-application mortality rate.

Romo-Martínez et al. (Boophilus) microplus (Acari: Ixodidae) in artificially infested bovine animals, demonstrated the efficacy of the fungus on Rhicephalus (Boophilus) microplus by reducing the number of larvae, nymphs and adults of this tick, from 7 to 21 days after fungal inoculation. These studies coincide with the present, regarding the efficacy of the strain MM01 of Metarhizium anisopliae to the concentration 1X108, but not with the commercial strains of Metarhizium anisopliae and Beauveria bassiana to the concentration 1.3 X 1012, which varies depending on the strain and the concentration used. According to Frazzon et al. (2000), Kirkland et al. (2004) and R.A. Campos, et al. (2010), this difference in efficacy depends on the ability of the fungus strain to penetrate the cuticle of the tick, using a combination of enzymatic and physical mechanisms. However, although there is a considerable amount of information on studies conducted with Metarhizium anisopliae for the control of Ricephalus (Boophilus) microplus ticks, the same is not true for the fungus of Beauveria bassiana, of which, with the exception of the work published by Ren et al. (2011), which evaluates the virulence of several Chinese strains of this fungus, there is little specific information published for the control of Ricephalus (Boophilus) microplus ticks.

Conclusions

The use of entomopathogenic fungi is an alternative for the control of adult ticks in the Maya XIII region of Chiapas and the Ríos region of the state of Tabasco, Mexico.

Metarhizium anisopliae and Beauveria bassiana are pathogenic in the adult state of Ricephalus (Boophilus) microplus ticks.

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