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Research article

Control of *Haematobia irritans* and *Stomoxys calcitrans* with *Metarhizium anisopliae* in naturally infested cattle

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ABSTRACT

Objective. Assess the efficacy of *Metarhizium anisopliae* strain 135 (Ma135) applied by aspersion to reduce simultaneous infestation of *Haematobia irritans* and *Stomoxys calcitrans* in naturally infested cattle maintained in a production system which combine grazing and confinement in pens. **Materials and methods**. Was applied an aqueous formulation of Ma135 ($1x10^8$ conidia/ml) on a nine cows group in six occasions with seven days interval, while the control group received the same formulation without conidial content. The infestation index was estimated daily for each fly independently; the effectiveness of the formulation was calculated using the Abbott's formula. **Results**. The Ma135 formulation had a reduction in the fly population of 58% for *H. irritans* and 69% for *S. calcitrans,* taking into consideration the six study weeks, in addition to causing no negative effects on animal health. An Infestation index reduction was observed from the first week post-treatment ($p<0.05$) and maintained this trend throughout the study. **Conclusions**. The present study has demonstrated the potential of strain Ma135 to reduce the simultaneous infestation of both hematophagous flies in cattle under conditions of natural infestation.

Keywords: Biological control; entomophatogenic fungi; horn fly; stable fly (*Source: DeSC, CAB*).

RESUMEN

Objetivo. Evaluar la eficacia de la cepa 135 de *Metarhizium anisopliae* (Ma135) aplicada por aspersión para reducir la infestación simultánea de *Haematobia irritans* y *Stomoxys calcitrans* en ganado naturalmente infestado mantenido en un sistema de producción de leche que combina el pastoreo y el confinamiento en corrales. **Materiales y métodos.** Se aplicó una formulación acuosa de Ma135 ($1x10^8$ conidios/ml) a un grupo de nueve vacas en seis ocasiones con un intervalo de siete días, mientras que el grupo control recibió una solución compuesta por agua, Monooleato de polioxietileno sorbitán en solución 0.01% y un adyuvante agrícola al 0.1%. Se estimó diariamente el índice de infestación para cada mosca de forma independiente; la efectividad de la formulación se calculó usando la fórmula de Abbott. **Resultados.** La formulación de Ma135 tuvo una eficacia en el control de la infestación de 58% para el caso de *H. irritans* y de 69% para *S. calcitrans*, tomando en cuenta las seis semanas de estudio, además de que no causó ningún efecto negativo en la salud de los animales. La reducción del índice de infestación se observó desde la primera semana post-tratamiento ($p<0.05$) y mantuvo esta tendencia durante todo el estudio. **Conclusiones.** El presente estudio ha demostrado el potencial de la cepa Ma135 para reducir la infestación simultánea de ambas moscas hematófagas en el ganado bajo condiciones de infestación natural.

Palabras clave: Control biológico; hongos entomopatógenos; mosca de los cuernos; mosca del establo (*Fuente: DeCS, CAB*).

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INTRODUCTION

Infestations by hematophagous flies represent a major health and economic problem in livestock farming, both for milk and meat production; the most important hematophagous flies in livestock are the stable fly, *Stomoxys calcitrans* and the horn fly, *Haematobia irritans* both are widely distributed in the American Continent and are responsible for significant losses in production as well as being efficient vectors of different microorganisms; the main damages in the cattle are caused due his hematophagous habits which cause direct effects by the loss of blood in addition to the alterations that this activity causes in the behavior and welfare of the animals, as well as, the loss of energy that causes to repel the attacks of these insects. These damages vary in their importance depending on the intensity of the infestation, but their effects are evidenced by the a decrease in milk production and weight gain, in addition to expenses incurred when applying chemical, physical or biological control measures (1,2).

H. irritans affects cattle in grazing since this production system favors its biological cycle; instead, *S. calcitrans* is a pest of cattle kept in confinement, in dairies and feedlots; however, when livestock are kept in production systems that combine grazing and housing, as well as in grazing systems with supplementation practices that favor reproduction of the stable fly, both flies may be present parasitizing livestock (1,2,3).

Hematophagous flies infestation is mainly controlled by the use of insecticides from different chemical families under various formulations; however, its widespread use without adequate technical advice has led to the emergence of resistance in several populations both in *H. irritans* and in *S. calcitrans*. The application of insecticides also has an impact on the environment and public health, so it is advisable to reduce their use in the production of food of animal origin (4,5,6,7).

Biological control using entomopathogenic fungi represents an alternative non-chemical control with minimal risk to the environment, vertebrates and humans (8); *Metarhizium anisopliae* sensu lato (s.l) is an entomopathogenic fungus that has demonstrated its efficacy as a biocontrol agent in *H. irritans* as well as in *S. calcitrans*, both in immature and adult stages under *in vitro* conditions (9,10,11,12,13), in the same way under conditions of natural and controlled infestation (11,14,15,16).

The objective of the present work was to assess the efficacy of Ma135 strain applied by aspersion to reduce simultaneous infestation of *H. irritans* and *S. calcitrans* in naturally infested cattle maintained in a production system which combine grazing and confinement in pens.

MATERIALS AND METHODS

Study site. This work was developed in the livestock production unit of the Instituto Tecnológico El Llano Aguascalientes, located in a municipality of the same name in Aguascalientes, México. The site is at 2020 masl, with a dry semi-warm climate, summer rains and an average temperature of 15.5°C.

Entomopathogenic fungi. The strain Ma135 was isolated from lodging pens in a commercial dairy production unit located in Jesús María municipality, in Aguascalientes, Mexico, using the larvae of *Galleria mellonella* L. (Lepidoptera: Pyralidae) as reservoir host. Taxonomic identification was based on morphological criteria of the reproductive structures; this strain has shown high pathogenicity for *S. calcitrans* and *Musca domestica* adults when evaluated under laboratory conditions and form part of the entomopathogenic fungi collection of the Instituto Tecnológico El Llano Aguascalientes (13).

The strain was cultured in the laboratory according to the protocol previously described by Cruz-Vázquez et al (15). Ma135 strain was cultured on Saboraud Dextrose Agar (DIBICO, Mexico), enriched with 1% yeast extract containing 500 ppm chloramphenicol (Tecnofarma, Mexico) and incubated al 25±1°C for 21 days, in a 12:12 h light/darkness regime. Conidia were harvested by scraping and suspended in sterile distilled water containing 0.1% (v:v) of Polioxietilen Sorbitan Monooleato (Sigma-Aldrich Co., St. Louis, MO), and homogenized on a vortex mixer. Spore viability, which exceeded 98%, was determined by seeding 100 µl of conidial suspension on Saboraud Dextrose Agar and colonies were counted 48 h later. Mass reproduction was carried out on rice grains in accordance with the methodology outlined by Ángel-Sahagún et al (17).

Field trial. Two groups were formed with nine Holstein cows, which did not receive any chemical treatment to control the infestation by flies during the year prior to this trial. The management of the animals was as follows: between 8 am and 4 pm each group was maintained in an area of 5000 m^2 cultivated with Rye Grass pasture (*Lolium perenne*), water tanks for drinking and natural shade provided by various native trees, the groups were separated from each other by 500 m. At 4 pm, the animals were transferred to the milking parlor and once finished the same they were housed in open pens, with dirt floors, shaded area, and concrete drinking and feed troughs, providing 40m^2 per cow. Each group were housed in different lodging pen, which had 500 m^2 of surface each and were separated from each other by 50 m; the cows received daily a nutritional supplement after the afternoon milking.

The study lasted 42 days (six weeks), starting the second week of August and ending the third week of September. The first group was treated with the aqueous formulation of Ma135, composed of 1 x 10^8 conidia/ml, water plus Polioxietilen Sorbitan Monooleato (Sigma-Aldrich Co., St. Louis, MO, USA) (0.01%) and an agricultural adjuvant at 0.1% (Inex-A, Cosmocel, Mexico). Each animal received the application of five liters of the formulation, prepared 30 min prior to administration, using a back sprayer with a cone-type nozzle at a pressure of approximately 40 lb^2, in six occasions with seven-day intervals between treatments; application intervals were similar to that used in our previous studies (15,16). The control group received the same formulation without conidial content. Applications were carried out from 7 to 8 pm to avoid the exposure to sunlight. The committee on Use and Care of Animals of the Instituto Tecnológico El Llano Aguascalientes approved this project, and adequate veterinary care was provided to all of the animals under study.

Estimation of *H. irritans* infestation. An infestation index (average number of flies per animal) was estimated daily by the direct count of adult flies found resting or feeding on the animals as described by Cruz-Vázquez et al (16), which consists of photographing only one side of the body, including regions of the head, neck, back, sides and limbs, using a digital camera with a 5x optical zoom function. This activity was done daily between 8 and 9 am, always by the same person, who was not aware of the status of the cows. Counting the number of flies was performed by computer image analysis. The obtained number was multiplied by two to obtain an estimate of the total number of flies; each cow was considered an experimental unit.

Estimation of *S. calcitrans* infestation. Infestation was estimated daily by direct counting of adult flies found resting or feeding on the front of the legs of the animals, by lateral observation, with the aid of binoculars when necessary (15); counting was done between 2 and 4 pm, always by the same person, who was not aware of the status of the cows; each cow was considered an experimental unit.

Climatic factors. This information was recorded as reported by the weather station of El Llano, Aguascalientes; the measurements included the average daily temperature (T°C), the average daily relative humidity (RH %) and the rainfall (Rf) during study period.

Statistical analysis. The data generated during the study period were analyzed using ANOVA and Student's-T test ($p<0.05$) in order to detect differences between groups each week using the corresponding week's average infestation index values; these calculations were developed for *H. irritans* and *S. calcitrans* independently. Efficacy of Ma135 formulation was estimated applying Abbott's formula in order to identify the percentage of reduction of the average infestation index for each evaluated week (14-16).

RESULTS

The aqueous formulation of Ma135 showed an efficacy to control the natural infestation by *H. irritans* in the cattle under study of 58%, reducing weekly infestation index from 357 to 150 in the six weeks of the trial. The weekly infestation index was statistically different between the groups in each of the six weeks of study ($p<0.05$); the effectiveness of the formulation was more important as the applications happened, being greater as more applications accumulated (Table 1). The efficacy of the formulation in the treated cattle was observed from the first week after its application and maintained the trend of reduction of the infestation index throughout the study, while the control group had an infestation index always greater than that of the treated group (Figure1). The population of *H. irritans,* on the day before treatment was estimated with an infestation index of 377 flies/animal in the treated group and 364 in the control group; the last day of the trial the control group had an Infestation index of 275 and the treated of 135.

The aqueous formulation of Ma135 was efficient to control the natural infestation by *S. calcitrans* in the cattle under study in 69%, reducing weekly infestation index from 44 to 13 in the six weeks of the trial. The weekly infestation index was statistically different between the groups in each of the six weeks of study ($p<0.05$); the effectiveness of the formulation was more important as the applications happened, being greater as more applications accumulated (Table 2). The efficacy of the formulation in the treated cattle was observed from the first week after its application and maintained the trend of reduction of the infestation index throughout the study, while the control group had an infestation index always greater than that of the treated group (Figure 1). The population of the stable fly, *S. calcitrans*, on the day before treatment was estimated with an infestation index of 46 flies/animal in the treated group and 44 in the control group; the last day of the trial the control group had an infestation index of 26 and the treated of 10. The cattle remained healthy during the study period and no signs of adverse reactions were observed at the local or systemic level.

Table 1. Infestation index average and efficacy of the Ma135 aqueous formulation applied on Holstein cows naturally infested with *H. irritans.*

Study week	Control group	Treated group	Difference[1]	Efficacy (%)[2]
1	398[a]	357[b]	41	10.16
2	404[a]	274[b]	130	32.14
3	373[a]	250[b]	123	33.14
4	424[a]	268[b]	156	36.87
5	333[a]	178[b]	155	46.59
6	283[a]	150[b]	133	47.13

Different literal in the same line (a, b) indicate significant differences ($p<0.05$). [1]Difference in the infestation index average between control and treated groups. [2]Reduction percentage on the average infestation index.

Table 2. Infestation index average and efficacy of the Ma135 aqueous formulation applied on Holstein cows naturally infested with *S. calcitrans*.

Study week	Control group	Treated group	Difference[1]	Efficacy (%)[2]
1	51a	44b	7	15.27
2	54a	39b	15	28.57
3	35a	23b	12	33.47
4	32a	21b	11	34.84
5	29a	16b	13	45.34
6	30a	13b	17	55.48

Different literal in the same line (a, b) indicate significant differences ($p<0.05$). [1]Difference in the infestation index average between control and treated groups. [2]Reduction percentage on the average infestation index.

Climatic conditions prevailing in the study period were as follows: average temperature was 19.9°C, with a range from 17.5 to 21.8°C; the beginning of autumn happened on day 40 of the test, however, the lowest daily average temperature occurred on day 35. The RH was on average 66.7%, with a range from 47 to 91%. During the study period, moderate rains occurred on days 15 and 16 of the trial (14.2 and 11.4 mm) and heavy rains on days 34 and 40 (29.8 and 23.9 mm).

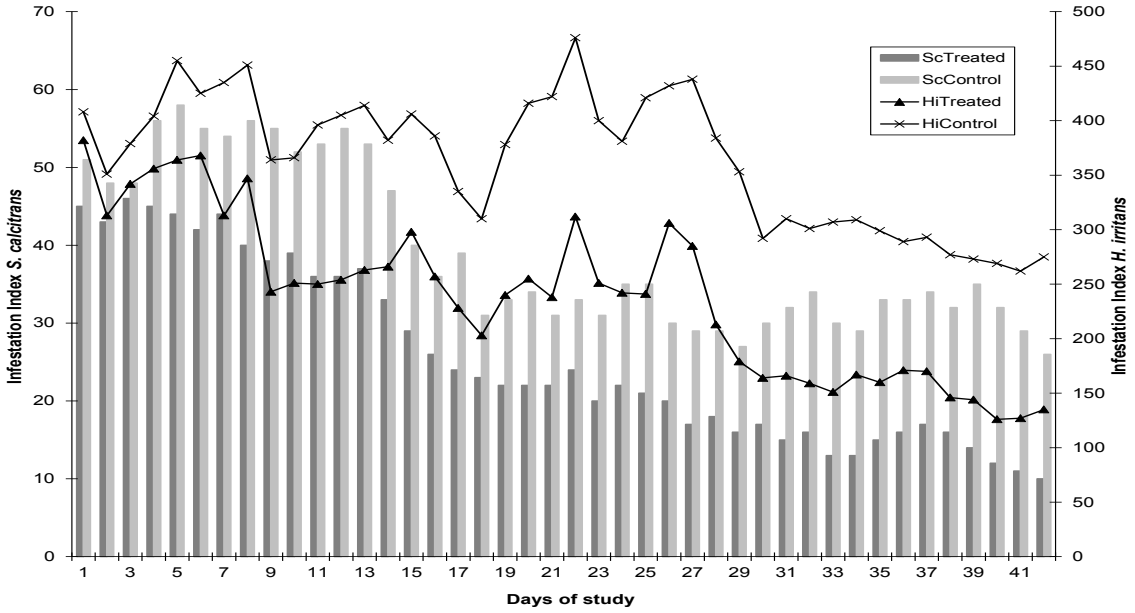

Figure 1. Daily infestation index of *S. calcitrans* (Sc) and *H. irritans* (Hi), in cows treated with the aqueous formulation of Ma135.

DISCUSSION

Infestation control of hematophagous flies in cattle using entomopathogenic fungi represents an alternative biological control that may help to limit the use of chemical insecticides and their negative effects on public health and the environment (5,8,18).

The results obtained in the present study have allowed to identify the capacity of Ma135 strain to control the natural infestation by both hematophagous flies in a system that combines grazing and confinement; this production model is frequently used in dairy farms to optimize resources. This trial had a duration of 42 days, beginning on August 12, with the idea of initiating the intervention before reaching the population peak reported for both flies in the region (15,16). The strain Ma135 showed an effectiveness in the six-week period of the trial of 58%, in the case of infestation caused by *H. irritans*, and 69% for infestation by *S. calcitrans*; the effectiveness of the formulation was evident from the first week post-treatment and maintained a tendency to reduce infestation index throughout the trial, which is also indicative of the additive effect of multiple application of the formulation, such as was already suggested in these hematophagous flies (15,16). No adverse effects were observed in the cattle after application of the formulation, a situation that has been reported by other studies developed in cattle infested with ticks or flies that were treated with formulations of *M. anisopliae* (15,16,19,20).

There is no information in the literature about the use of an entomopathogenic fungi formulation for the simultaneous control of *H. irritans* and *S. calcitrans* under natural infestation conditions; however, different aqueous formulations of *M. anisopliae* has been evaluated under natural and controlled infestation conditions of *H. irritans* with promising results. Mochi et al (10), applied by aspersion an aqueous formulation of *M. anisopliae* E9 (3×10^{10} conidia/ml), four times with interval of 5 days to eight animals naturally infested with *H. irritans*, reducing infestation above 50%. In other study, Galindo-Velasco et al (14), in a study with controlled infestation of *H. irritans* found a reduction of infestation of up to 100% at 13 days post-treatment, using different strains at a concentration of 1×10^8 colony forming units/ml applied by spraying. Recently, Cruz-Vázquez et al (16), studied the efficacy of aqueous formulation of Ma134 strain at a concentration of 1×10^8 conidia/ml, applied by aspersion in four occasions with seven days interval in Holstein cows naturally infested with *H. irritans*, in this study the reduction was 68.6%. In the case of *S. calcitrans*, there is only the evaluation of aqueous formulation of Ma134, applied by aspersion at a concentration of 1×10^8 conidia/ml four times with interval of seven days to eight animals naturally infested; in the trial the efficacy of aqueous formulation was 73% (15).

The efficacy of Ma135 was slightly lower than that reported previously with Ma134 strain in studies developed in the same experimental site by our group; however, the efficacy of aqueous formulation of Ma135 was higher than 55% and for a longer period of time (six weeks vs four weeks). The difference between studies may be due to the conditions in which the current trial was developed; among which we must mention the days of duration, number of applications, the population abundance curve of the flies that is related to the climatic conditions, and the variability to which the effectiveness of the formulation can be subject that depend on several environmental factors that have been previously discussed, such as solar radiation, temperature, rainfall and environmental humidity (15,16,21).

The formulation was designed to protect the conidia from the effect of UV light by incorporating the

agricultural adjuvant, in addition, the time of day when the formulation was applied also helped to protect the conidia; the adjuvant also allowed the adhesion of the conidia to the hair of the animals and to the body of the flies. The weather conditions prevailing in the period of the trial helped to maintain a high relative humidity with rainfall on four occasions, these precipitations did not coincide with the applications of formulated to cattle; we consider that the characteristics of the formulation and the climatic conditions present during the study period helped to promote the efficacy of the formulated. In addition, the Ma135 strain was isolated from the soil of the Aguascalientes state, and therefore must be adapted to the environmental conditions of the region. The dairy production unit from which the Ma135 strain was isolated is located 45 km from the Instituto Tecnológico El Llano Aguascalientes; the site is at 1890 masl, with a dry semi-warm climate, summer rains and an average temperature of 17.4°C. The population curve of the control group always maintained a higher infestation index than the treated group and its behavior was as expected according to the previous antecedents reported in the area and in the site where the study was developed (15,16).

The use of *M. anisopliae* represents a non-chemical control alternative with minimal risk to the environment, vertebrates and humans (8), that allows to limit the application of chemical insecticides, but does not eliminate the need for the production units to develop basic manure management and environmental sanitation work to prevent the development of the immature stages of the flies. In conclusion, the present study has demonstrated the potential of strain Ma135 prepared as an aqueous formulation with the addition of an agricultural adjuvant, to control the natural and simultaneous infestation of hematophagous flies *H. irritans* and *S. calcitrans* in cattle without causing any adverse effect on the animal's health.

Conflicts of interest

None of the authors have any conflict of interest in regard to this article.

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REFERENCES

1. Taylor D, Moon R, Mark D. Economic impact of stable flies (Diptera: Muscidae) on dairy and beef cattle production. J Med Entomol. 2012; 49:198–209. DOI: https://doi.org/10.1603/ME10050 PMid:22308789

2. Ibarra VF, Figueroa CJA, Quintero M. editores. Parasitología Veterinaria volumen III artrópodos. Universidad Nacional Autónoma de México; CDMX México; 2012. URL Disponible en: https://www.researchgate.net/profile/Gerardo_Rivas/publication/280491915_Introduccion_y_generalidades_de_los_Artropodos/links/55b65f5908aed621de0352fd/Introduccion-y-generalidades-de-los-Artropodos.pdf

3. Dominghetti FST, Barros ATM, Soares CO, Cançado PHD. Stomoxys calcitrans (Diptera: Muscidae) outbreaks: current situation and future outlook with emphasis on Brazil. Braz J Vet Parasitol. 2015; 24:387-395. DOI: https://doi.org/10.1590/S1984-29612015079 PMid:26689177

4. Kunz SE, Kemp DH. Insecticides and acaricides resistance and environmental impact. Rev Sci Technol. 1994; 13:1249–1286. DOI: https://doi.org/10.20506/rst.13.4.816

5. Oyarzún MP, Quiroz A, Birkett MA. Insecticide resistance in the horn fly: alternative control strategies. Med Vet Entomol. 2008; 22:188-202. DOI: https://doi.org/10.1111/j.1365-2915.2008.00733.x PMid:18816268

6. Pitzer JB, Kaufman PE, Tenbroeck SH. Assessing permethrin resistance in the stable fly (Diptera: Muscidae) in Florida by using laboratory selections and field evaluations. J Econ Entomol. 2010; 103:2258–2263. DOI: https://doi.org/10.1603/EC10166 PMid:21309252

7. Salem A, Bouhsira E, Lienard E, Bousquet MA, Jacquiet P, Franc M. Susceptibility of two European strains of Stomoxys calcitrans (L.) to cypermethrin deltamethrin, fenvalerate, k-cyalothrin, permethrin and phoxim. J Appl Res Vet Med. 2012; 10:249-257. URL Disponible en: https://www.jarvm.com/articles/Vol10Iss3/Vol10%20Iss3%20Franc.pdf

8. Zimmerman G. Review on safety of entomopathogenic fungus Metarhizium anisopliae. Biocontrol Sci Tech. 2007; 17:879-920. DOI: https://doi.org/10.1080/09583150701593963

9. Moraes AP, Angeloldac C, Fernandez EK, Bittencourt VR, Bittencourt AJ. Virulence of Metarhizium anisopliae to eggs and immature stages of Stomoxys calcitrans. Ann NY Acad Sci. 2008; 1149:384-387. DOI: https://doi.org/10.1196/annals.1428.008 PMid:19120256

10. Lohmeyer K, Miller J. Pathogenicity of three formulations of entomopathogenic fungi for control of adult Haematobia irritans (Diptera: Muscidae). J Econ Entomol. 2006; 99:1943-1947. DOI: https://doi.org/10.1093/jee/99.6.1943 PMid:17195658

11. Mochi DA, Monteiro AC, Simi LD, Moraes SA. Susceptibility of adult and larvae stages of the horn fly Haematobia irritans, to entomopathogenic fungus Metarhizium anisopliae under field conditions. Vet Parasitol. 2009; 166:136-143. DOI: https://doi.org/10.1016/j.vetpar.2009.07.037 PMid:19713044

12. Mochi DA, Monteiro AC, Ribeiro MAC, Yoshida L. Entomopathogenic fungal activity against pupae and adult Haematobia irritans (Diptera: Muscidae). Vet Parasitol. 2010; 168:105-110. DOI: https://doi.org/10.1016/j.vetpar.2009.10.002 PMid:19880254

13. López-Sánchez J, Cruz-Vázquez C, Lezama-Gutiérrez R, Ramos-Parra M. Effect of entomopathogenic fungi upon adults of Stomoxys calcitrans and Musca do mestica (Diptera: Muscidae). Biocontrol Sci Technol. 2012; 22:969-73. DOI: https://doi.org/10.1080/09583157.2012.699026

14. Galindo-Velasco E, Lezama-Gutiérrez R, Cruz-Vázquez C, Pesador-Rubio A, Ángel-Sahagún CA, Ojeda-Chi MM, et al. Efficacy of entomopathogenic fungi (Ascomycetes:Hypocreales) against adult Haematobia irritans (Diptera:Muscidae) under stable conditions in the Mexican dry tropics. Vet Parasitol. 2015; 2009:173-178. DOI: https://doi.org/10.1016/j.vetpar.2015.02.025 PMid:25771932

15. Cruz-Vázquez C, Carvajal-Márquez J, Lezama-Gutiérrez R, Vitela-Mendoza I, Ramos-Parra M. Efficacy of the entomopathogenic fungi Metarhizium anisopliae in the control of infestation by stable flies Stomoxys calcitrans (L.), under natural infestation conditions. Vet Parasitol. 2015; 212:350-355. https://doi.org/10.1016/j.vetpar.2015.07.003 PMid:26209932

16. Cruz-Vázquez C, Carvajal-Márquez J, Lezama-Gutiérrez R, Vitela-Mendoza I, Ángel-Sahagún CA. Efficacy of Metarhizium anisopliae in the control of the horn fly, Haematobia irritans (Diptera: Muscidae) under natural infestation conditions. Vet Mex OA. 2017; 4:2. URL Available in http://veterinariamexico.unam.mx/index.php/vet/article/view/384

17. Ángel-Sahagún C, Lezama-Gutiérrez R, Molina-Ochoa J, Pescador-Rubio A, Skoda SR, Cruz-Vázquez C, et al. Virulence of mexican isolates of entomopathogenic fungi (Hypocreales: Clavicipitaceae) upon Rhipicephalus=Boophilus microplus (Acari: Ixodidae) larvae and the efficacy of conidia formulations under field conditions. Vet Parasitol. 2010; 170:278-286. DOI: https://doi.org/10.1016/j.vetpar.2010.02.037 PMid:20359827

18. Ojeda-Chi MM, Rodríguez-Vivas RI, Galindo-Velasco E, Lezama-Gutiérrez R, Cruz-Vázquez C. Control de Rhipicephalus microplus (Acari: Ixodidae) mediante el uso del hongo entomopatógeno Metarhizium anisopliae (Hypocreales: Clavivipitaceae). Rev Mex Cienc Pecu. 2011; 2:177-192. URL Available in: http://cienciaspecuarias.inifap.gob.mx/index.php/Pecuarias/article/view/1445

19. Kaaya GP, Samish M, Hedimbi M, Gindin G, Glazeer I. Control of tick population by spraying Metarhizium anisopliae conidia on cattle under field conditions. Exp Appl Acarol. 2011; 55:273-281. DOI: https://doi.org/10.1007/s10493-011-9471-3 PMid:21725837

20. López E, López G, Orduz S. Control de la garrapata Boophilus microplus con Metarhizium anisopliae, estudios de laboratorio y campo. Rev Colomb Entomol. 2009; 35:42-46. URL Available in: http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0120-04882009000100008

21. Inglis GD, Duke GM, Goettel MS, Kabaluk JT. Genetic diversity of Metarhizium anisopliae var. anisopliae in southwestern British Columbia. J Invertebr Pathol. 2008; 98:101–113. DOI: https://doi.org/10.1016/j.jip.2007.12.001 PMid:18215399