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Short communication

Evaluation of *Metarhizium anisopliae* (Hyphomycetes) for the control of *Boophilus microplus* (Acari: Ixodidae) on naturally infested cattle in the Mexican tropics

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Abstract

The efficacy of *Metarhizium anisopliae* on the control of *Boophilus microplus* in cattle infested naturally in the Mexican tropics was evaluated. The study was carried out on a ranch in Veracruz, Mexico. Twenty steers were randomly allocated into two groups of 10 cattle. Animals were naturally infested with *B. microplus*. Animals in the treated group were sprayed with *M. anisopliae* (strain Ma34) at a concentration of 1×10^8 conidia/ml every 15 days (four treatments). The other group remained as untreated control. Standard engorged female ticks were recorded on days 0, 1, 3, 5, 7 and 14 post-treatment. From the second application treatment (day 7) to the end of the experiment, animals in the treated group had lower tick infestation ($P < 0.05$) with an efficacy of 40.0–91.2%. The results demonstrate the efficacy of repeated treatment with *M. anisopiae* (Ma34 strain) to control natural infestation of engorged female *B. microplus* on cattle in the Mexican tropics.

Keywords: *Metarhizium anisopliae*; *Boophilus microplus*; Cattle; Biological control; Entomopathogenic fungi; Tropics

1. Introduction

The tick *Boophilus microplus* is a bovine ectoparasite that causes economic losses in herds of tropical and subtropical areas of Mexico, due to the diseases it transmits and the parasitism itself, which causes reduction in milk yield, calf production and high costs to control the tick (Rodríguez-Vivas and Domínguez-Alpizar, 1998).

Control strategies for tick population rely to a large extent on the use of chemical acaricides such as organophosphates, pyrethroids and amidines (Rodriguez-Vivas et al., 2006). The need for alternative methods to control tick populations in Mexico has been stimulated due to the increased tick resistance (Alonso-Díaz et al., 2006), and the fact that chemical acaricides can lead to environmental damage and are often toxic to humans as well as other non-target organisms (Kirkland et al., 2004). Biological control methods for ticks

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include the promotion of natural predators such as beetles, spiders, and ants. The use of tick parasites ranges from insects to nematodes (Samish and Rehacek, 1999). Biological control based on entomopathogenic fungi is one of the most promising options to control ticks (Polar et al., 2005). The fungi *Metarhizium anisopliae* has been extensively studied as a key regulatory organism for biocontrol (Frazzon et al., 2000; Dutra et al., 2004). *M. anisopliae* invades *B. microplus* by a process which involves the adhesion of conidia to the cuticle, conidia germination, formation of appressoria and penetration through the cuticle, observing a massive penetration 72 h post-inoculation (Arruda et al., 2005). In Mexico, some strains of *M. anisopliae* have been isolated with promising results to control *B. microplus* in laboratory conditions (Ojeda, 2007). The *M. anisopliae* Ma34 strain showed a 100% efficacy on eggs and adults of *B. microplus* when it was used at 10^8 conidia/ml. However, in Mexico its efficacy on field conditions has not been investigated. The aim of the present study is to evaluate the efficacy of *M. anisopliae* for the control of *B. microplus* in cattle infested naturally in the Mexican tropics.

2. Materials and methods

2.1. Study area

The present study was carried out in Martinez de la Torre, Veracruz, Mexico (Centro de Enseñanza, Investigación y Extensión en Ganadería Tropical-FMVZ-UNAM). The climate of the zone is humid tropical, with mean annual temperature of 24.4 °C, annual rainfall of 1990 mm and relative humidity (RH) of 85% (García, 1981). The study was conducted between April and July 2005. High *B. microplus* infestation on cattle was reported in the studied area and the maximum tick burden occurred between May and July (Alonso-Díaz et al., in press).

2.2. *M. anisopliae* strain and culture conditions

M. anisopliae strain (Ma34) was originally isolated from soil of Tocumbo Michoacan, Mexico in the year 2000 by our research group. The fungus was deposited in the mycological collection of the University of Colima, Mexico. The fungus was grown on Sabouraud dextrose agar (SDA) and 1% yeast extract on tube (Moorhouse et al., 1993) with 500 ppm of chloramphenicol (Sneh, 1991), incubated at 25 ± 1 °C (Barson et al., 1994) and 70% RH for 3 weeks. Conidia were harvested by scraping and suspending in sterile distilled water containing 0.1% (v/v) Tween 80, and the suspension was poured into a sterile glass tube and homogenized on a vortex mixer.

Conidial concentrations were determined by direct count using an improved Neubauer haemocytometer and adjusted to 10^8 conidia/ml by dilution with 0.1% Tween 80. One milliliter of the suspension was taken and diluted to 10^6 conidia/ml using 0.1% Tween 80. Spore viability was determined by placing 100 μl of the conidial suspensions on Sabouraud dextrose agar and counting colonies 48 h later (Lacey et al., 1994). Spore viability exceeded 98%.

The fungus was produced on rice grain. In a previous study, rice was found to be the best media for mass-culturing the fungi for field studies (Lezama and Munguía, 1990). Polyethylene bags containing 200 g of rice (Milagro Filipino) were soaked in distilled water with 500 ppm of chloramphenicol for 40 min, and autoclaved for 15 min at 121 °C and 15 psi. Each bag was inoculated with 1 ml of the suspension (10^8 conidia/ml) by using a sterilized syringe. The puncture was sealed with sticky tape. Spores were left for incubation for 3 weeks in the dark at 25 °C. The suspension was adjusted to a final concentration of the 10^8 UFC/ml before evaluation on field conditions.

The spores of *M. anisopliae* (strain Ma34) were selected and sprayed with water plus Tween 80 on bovine using 5 l for animals (to cover all animal body) for evaluation against *B. microplus* adult ticks on natural conditions.

2.3. Experiment design

Twenty steers (Holstein × Zebu) with 18 ± 4 (standard deviation) months of age and 255.7 ± 49.7 (standard deviation) kg were used in the experiment. The animals were naturally infested with *B. microplus*. The general pasture area used in the study consisted of individually fenced 5 ha pastures.

Primary forage during the time of the study consisted of African star grass (*Cynodon nlemfuensis*), native forage (*Axonopus* spp. and *Paspalum* spp.) with fresh water and minerals *ad libitum*. Food supplementation (16% crude protein) at a rate of 1% of body weight/day was provided to all animals. Ninety days previous and during the experiment acaricide treatment to control ticks was not applied.

On day 0 the animals were ranked on the basis of standard counts engorging female ticks of 4.5–8.0 mm (Wharton and Utech, 1970) to form two similar groups with 10 cattle. The total number of female ticks was obtained by multiplying the number of female ticks of the left side of each animal per two. All animals were identified with ear tags and each group of animals was then released on the same pasture into two similar fenced off parts for the duration of the experiment.

Animals in group one (control group) were sprayed with water plus Tween 80 (1 ml Tween/1 l of water) of suspension using a back sprayer with a cone-type nozzle and a pressure of about 40 lb/in^2. Animals in group two were sprayed with *M. anisopliae* (strain Ma34) at a concentration of 1×10^8 conidia/ml (also 0.1% Tween 80 was used). All treatments were applied on days 0, 15, 30 and 45. Animals were treated in the afternoon (6–7 p.m.) to avoid sunlight (Polar et al., 2005). According to literature we assumed that *M. anisopliae* persisted on cattle for 3 weeks (Kaaya et al., 1996).

Standard counts of engorged female ticks were recorded on days 0, 1, 3, 5, 7 and 14 post-treatment (PT) and a sample of ticks was sent to the parasitology laboratory for tick classification following the keys of Rodriguez-Vivas and Cob-Galera (2005). Cattle in the trial were observed on the day of treatment and at each inspection for evidence of any adverse reactions.

2.4. Data analysis

For each time of measurement, tick counts of control and treated groups were compared using the U Mann Whitney test to determine whether or not treatment effects were significant (SAS, 1991).

The efficacy of *M. anisopliae* treatment was evaluated using a formula described by Morin et al. (1996):

$$\text{efficacy}\ (\%) = \frac{(\text{mean tick no. on control group}) - (\text{mean tick no. on treated group})}{\text{mean tick no. on control group}} \times 100$$

3. Results

There was no evidence of any local or systemic adverse reaction in treated animals and all cattle remained healthy throughout the experiment. The number of ticks and efficacy of the entomogenous fungi *M. anisopliae* against natural infestation of engorged female *B. microplus* is shown in Table 1. From the second application treatment to the end of the experiment, animals in the treated group had lower tick infestation ($P < 0.05$) (except at the end of the third treatment). In the third treatment, animals in the control group had the maximum *B. microplus* infestation (average of 243 engorged females) conversely with 30 engorged female in the control group. More than 45% of efficacy was observed from the second application treatment to the end of the experiment (except at the end of the third treatment).

Table 1
Number of ticks and efficacy of the entomogenous fungi *M. anisopliae* strain Ma34 against natural infestation of engorged female *B. microplus* (4.5–8.0 mm) on cattle in the Mexican tropics

No. of dips	Days	Average number of engorging female ticks		Efficacy (%)
		Control group	Treated group	
1	0	10.6 a	7.2 a	32.1
	1	7.8 a	8.2 a	1
	3	6.2 a	3.4 a	45.2
	5	4.2 a	2 a	52.4
	7	4.2 a	3.8 a	9.5
	14	3 a	4.6 a	0.0
2	1	2.4 a	2.6 a	0.0
	3	2.8 a	1.6 a	42.9
	5	6.8 a	6.4 a	5.9
	7	19 a	8 b	57.9
	14	95.6 a	23.2 b	75.7
3	1	135.4 a	16.2 b	88.0
	3	243 a	30.6 b	87.4
	5	174.6 a	29.4 b	83.2
	7	135 a	26.2 b	80.6
	14	15 a	9 a	40.0
4	1	18.4 a	10 b	45.7
	3	14.6 a	5.2 b	64.4
	5	25 a	2.2 b	91.2
	7	20.2 a	2.4 b	88.1
	14	6.6 a	1.6 b	75.8

Values with different letters in the same rows differ at $P < 0.05$.

4. Discussion

Entomogenous fungi have several advantages as low environmental pollutant, low risk for animals and high efficacy against pests. *M. anisopliae* has been considered one of the most promising agents for biological control of ticks. *In vitro* studies reported that *M. anisopliae* is highly infective to engorged females (Frazzon et al., 2000; Onofre et al., 2001), larvae (Bahiense et al., 2006) and nymphs (De Moraes et al., 2003) of *B. microplus*. The *M. anisopliae* Ma34 strain showed a 100% efficacy on eggs and adults of *B. microplus* when it was used at 10^8 conidia/ml under laboratory conditions in Mexico (Ojeda, 2007).

Under field conditions the efficacy of *M. anisopliae* on *B. microplus* can be poor due to several factors. In pen trails in Brazil by Correia et al. (1998), there was no

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notice of any significant effect on the number of female parasiting bulls with a single spray of *M. anisopliae* after 16 days of evaluation. However, when females were transferred 1 day after treatment to laboratory conditions, the fungus had grown and sporulated (91.7% of females collected from the bulls) causing high mortality. In the present study, we demonstrated that an efficient control ($P < 0.05$) of *B. microplus* on naturally infected cattle was achieved by the Ma34 isolation and this effect was shown after the second fungal application (after 7 days of treatment). Hornbostel et al. (2004), mentioned that *M. anisopliae* reduces fitness (fecundity and body mass) in all active *Ixodes scapularis* stages and indicates that its impact as a biocontrol agent might be higher than that suggested by direct mortality alone.

In the present study *M. anisopliae* Ma34 strain showed >45% of efficacy to control standard female ticks (from the second application treatment to the end of the experiment). This finding is in agreement with De Castro et al. (1997) who recorded in pen trials a decrease of >50% in the *B. microplus* population treated with another *M. anisopliae* isolate.

On field condition, several host microclimatic factors may influence the pathogenicity of entomopathogenic fungi to ticks on the cattle surface. The adverse effects of UV-A and UV-B radiation and heat from sunlight are major environmental factors negatively affecting the field use of entomopathogens as biocontrol agents (Polar et al., 2005; Rangel et al., 2005; Iskandarov et al., 2006). Recently, Andersen et al. (2006) reported that their efficacy can be poor in environments where water availability is reduced. Polar et al. (2005) found that the greater control of ticks on pasture grazed cattle was achieved by a *M. anosopliae* isolate that was more tolerant to higher temperatures (31–35 °C). Similar results were reported by Rangel et al. (2005) who suggest that the high stability to UV-B tolerance of *M. anisopliae* (ARSEF 324 strain) is due to strong natural selection to survive the harsh climate of its original collection site (Rangel et al., 2005). In our study the conidia treatment was applied at 6–7 p.m. when the temperature ranged 28–32 °C in the absence of sunlight. During the night the superficial temperature of cattle is near 28 °C and the diurnal temperature fluctuation was considered to be more biologically relevant for *M. anisopliae* (Polar et al., 2005). Further studies to evaluate the efficacy of *M. anisopiae* Ma34 strain to control *B. microplus* on field conditions in different microclimates are needed. Furthermore, it is important to carry out studies on UV-B-stress tolerance of the Ma34 strain.

In the present study, from the second application treatment to the end of the experiment, animals in the treated group had lower tick infestation ($P < 0.05$) (except at the end of the third treatment). Chronological histological alterations of *M. anisopliae* during interaction with the cattle tick *B. microplus* were investigated by light and scanning electron microscopy (Arruda et al., 2005). A massive penetration was observed 72 h post-inoculation, and after 96 h the hyphae start to emerge from the cuticle surface to form conidia (Arruda et al., 2005).

The results obtained in this study, demonstrate the efficacy of repeated treatment with *M. anisopiae* (Ma34 strain) to control natural infestation of engorged female *B. microplus* on cattle in the Mexican tropics.

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