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Short communication

Metarhizium anisopliae for controlling *Rhipicephalus microplus* ticks under field conditions

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ABSTRACT

Metarril SP Organic is a product based on the fungus *Metarhizium anisopliae*, which was developed for controlling agricultural pests. The present study evaluated the effect of Metarril SP Organic plus 10% mineral oil, for controlling *Rhipicephalus microplus* under field conditions. Three groups were formed: Control group, which received no treatment; Oil control group, which was bathed only with water, Tween 80 and mineral oil; and Metarril group, bathed in the oil-based formulation Metarril SP Organic. Two treatments per group were performed and to verify the effect of the treatments, all *R. microplus* ticks between 4.5 and 8.0 mm in length on the left side of the cattle were counted on days +7, +14 and +21 after each treatment, and a sample of engorged females was collected for evaluation of biological parameters. The Metarril SP Organic oil formulation showed efficacy ranging from 8.53 to 90.53%. The average efficacy of the oil-based formulation of Metarril SP Organic was 75.09 and 46.59% compared with the groups Control and Oil control, respectively. There were no significant changes in biological parameters of engorged *R. microplus* females collected from animals. Although there was no significant difference in the amount of ticks between the Oil control and Metarril groups, it is believed that the association of mineral oil with Metarril SP Organic product is effective in *R. microplus* tick control in field. Thus, this association has potential to be used in strategic control programs of cattle tick.

1. Introduction

Interest in biological control programs for agricultural pests and parasites in livestock has grown considerably around the world because of new directives for production that promotes conservation and sustainable use of biological resources, in addition to concerns about the food quality and the absence of chemical residues in meat and dairy products. However, the use of chemical insecticides and acaricides is still the predominant method for controlling pests and parasites such as *Rhipicephalus microplus* ticks. This parasite has a major economic impact on livestock because of the direct and indirect losses it causes. It has been estimated that in Brazil alone, these losses reach approximately 3.24 billion dollars a year (Grisi et al., 2014).

Lack of concern about proper use of the chemical bases that are commercially available has reduced the effectiveness of these products through selection and spreading of resistant ticks (Furlong et al., 2007). In this context, biological control using arthropod-pathogenic fungi is a promising method that should be considered in relation to tick control.

The virulence of these fungi against ticks has already been proven in many laboratory studies (Fernandes et al., 2006; Leemon and Jonsson, 2008; Camargo et al., 2012; Quinelato et al., 2012). However, under natural conditions, this virulence decreases because the action of these pathogens is influenced by various

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environmental factors, such as temperature, relative humidity and exposure to solar radiation (Butt et al., 2001; Leemon et al., 2008; Ojeda-Chi et al., 2010; Fernandes et al., 2012). Therefore, it is necessary to evaluate the action of fungi under environmental conditions in order to devise ways of using these pathogens without losing their infectivity and virulence.

The formulation in which the conidia are applied is of fundamental importance to the success of tick biocontrol because this maintains the viability, virulence and effectiveness of the pathogens under field conditions. Some products composed by arthropod-pathogenic fungi were tested against different tick species under laboratory conditions. Oil formulations of a product based on isolates ESALQ 1037 and ESALQ E9 of *Metarhizium anisopliae* (Metarril WP Organic) were tested against *R. microplus* tick and presented significant changes in all evaluated parameters (Marciano et al., 2013). The products Metarril SC (this product is no longer available on market) and Boveril WP were tested against *Amblyomma cajennense* tick (Lopes et al., 2007). Boveril WP was also tested against *R. microplus* (Perinotto et al., 2012a) and *Dermacentor nitens* (Perinotto et al., 2012b). All these products referred to above caused significant changes against ticks tested, especially when elaborated in oil formulations.

The present study was developed through motivation from the results found by Camargo et al. (2014). In assessing the oil-based formulation of Metarril SP Organic, a product based on *M. anisopliae* fungus, against cattle ticks in a pen-based study, these authors concluded that this oil-based fungal formulation might be an important tool for controlling *R. microplus* ticks, and that studies in the field using more than one treatment would be beneficial for increasing the effectiveness of this product for tick control under natural conditions. Therefore, the aim of the current study was to evaluate the formulation of Metarril SP Organic, plus 10% mineral oil, for controlling *R. microplus* ticks in field tests.

2. Material and methods

2.1. Location and period of the experiment

The field test was carried out between the months of April and June 2014, in the field area of the "W.O. Neitz" Experimental Station for Parasitological Research of the Federal Rural University of Rio de Janeiro (UFRRJ), Seropédica campus, Rio de Janeiro, Brazil, which is located at 22° 44′ South, 43° 42′ West. This region has a tropical climate, an altitude of 33 m, average annual temperature of 23.5 °C and average annual rainfall of 1483 mm. The evaluation of the biological parameters of *R. microplus* females collected from animals during the test was carried out from May to July 2014 in the Microbial Control Laboratory of UFRRJ.

2.2. Cattle used in the experiment

A total of 30 Angus cattle, Red Angus variety, aged approximately between 1 and 4 years (mean age 1.87 years) and weighing between 155 and 461 kg (average weight of 313.1 kg), were used. The groups were kept in three separate paddocks, with an area of about 2 ha per paddock with grass and water freely available. This study was approved by the Ethics Committee for Animal Use (CEUA) of UFRRJ, under the protocol number 017/2014.

2.3. Formulations evaluated in the experiment

A formulation only containing water, 10% mineral oil (Vetec Fine Chemicals Ltda., Rio de Janeiro, RJ, Brazil) and 1% Tween 80 was prepared for the treatment administered to the oil control group. A fungal formulation was prepared using Metarril SP Organic, water, 10% mineral oil and 1% Tween 80. The product Metarril SP Organic was developed by Koppert Biological Systems to control agricultural pests and consisted of the isolates ESALQ 1037 and ESALQ E9 of *M. anisopliae*. The fungal formulation used in the field test contained 1×10^8 conidia/mL.

2.4. Experimental design

All the methodology used in the field test was performed in accordance with to the methodology described by Holdsworth et al. (2006) and the methodology recommended by the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA) for production, control and use of antiparasitic drugs for veterinary purposes (Ordinance No. 48 of May 12, 1997; MAPA) (Brasil, 2012).

The animals used in the field test were naturally infested with *R. microplus* ticks, i.e., all animals had sufficient natural parasite load for the field test development, not being necessary the artificial infestation of animals with tick larvae (Holdsworth et al., 2006). Before the experiment was started, ticks between 4.5 and 8.0 mm long were counted weekly so as to assure the presence of at least 20 ticks on one side of each animal. The 30 cattle were distributed among the groups according to the infestation degree, determined according to the number of ticks between 4.5 and 8.0 mm present on one side of the body of each animal over the three days prior to treatment (−3, −2 and −1). The three animals with the highest average tick counts were randomly allocated, one to each group. The next three animals were then allocated, and so on until the three groups of ten animals each had been completed (Holdsworth et al., 2006). Thus, the groups began the test with very similar average number of ticks.

Two treatments with the formulations were performed on the cattle: the first treatment on day zero and the second on day +3. There were three test groups: a group treated with the oil-based formulation of Metarril SP Organic (Metarril); a group treated only with a formulation of water, mineral oil and Tween 80 (Oil Control) and a control group that received no treatment (Control). Using a backpack pump sprayer system, the formulations were sprayed over the animals' bodies, from the bottom up and in the opposite direction to how the fur lies, giving greater attention to the areas most affected by ticks, such as the inner thighs, dewlap, ears and perineum. Each animal was sprayed with 4 L of the corresponding formulation, which was enough to bathe the entire body surface of an animal with the physical characteristics listed above.

After the cattle had been treated with the formulation, all female ticks between 4.5 and 8.0 mm in length, present on the left side of each animal were counted on days +7, +14 and +21 after each treatment, i.e. a total of six counts (+7, +10, +14, +17, +21 and +24). A sample of ten engorged females per group per day was randomly collected to evaluate the biological parameters. They were taken to the laboratory, individually weighed, fixed in Petri dishes and incubated at 27 ± 1 °C and relative humidity ≥80%. The eggs produced by the females were weighed and incubated under the same temperature and humidity conditions for further evaluation of the larvae that hatching.

To evaluate the efficacy of the oil-based formulation of Metarril SP Organic on *R. microplus* ticks in the field tests, the following biological parameters were investigated: initial weight of the female, weight of the egg mass, larvae hatching percentage, and nutritional and egg production indexes (Bennett, 1974).

The efficacy of the Metarril SP Organic formulation was calculated using the following formula (Henderson and Tilton, 1955; Holdsworth et al., 2006; Brasil, 2012):

$$\text{Efficacy} = \left(1 - \frac{\mathbf{T_a} \times \mathbf{C_b}}{\mathbf{T_b} \times \mathbf{C_a}}\right) \times 100 \quad (1)$$

where $\mathbf{T_a}$ = average number of ticks that fell from treated animals after the day of treatment; $\mathbf{T_b}$ = average number of ticks that