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Efficiency of *Lecanicillium lecanii* to control the tick *Rhipicephalus microplus*

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ABSTRACT

Rhipicephalus microplus, known as the cattle tick, causes serious economic losses in the Brazilian cattle industry each year. Traditional parasite control is primarily based on the use of chemical acaricides, which unfortunately have many negative side effects. Biological control is seen as a promising alternative to chemical acaricide use. This study evaluates the entomopathogenic fungus *Lecanicillium lecanii* for effectiveness in controlling engorged females, eggs, and larvae of *R. microplus*. Conidial formulations of *L. lecanii*, isolate CG 420, were prepared in both oil (15% mineral oil) and aqueous suspensions. Ticks were immersed in a 1 ml oil-based conidial suspension at 1×10^8 conidia ml^{-1} or one of several aqueous conidial suspensions at 1×10^5, 10^6, 10^7 or 10^8 conidia ml^{-1}. The control groups were immersed in water or oil solutions with no conidia. Treatments with aqueous conidial suspensions were conducted with 10 ticks per group (1×10^5, 10^6, 10^7, 10^8 conidia ml^{-1} or control) whereas the oil treatments used 30 ticks per group (1×10^8 conidia ml^{-1} or control). Bioassays were repeated twice on different days with different batches of conidia. After treatment, the biological parameters of engorged females were evaluated, while eggs and larvae were evaluated taking into consideration hatchability and mortality, respectively. The results indicate that *L. lecanii* has the potential to control engorged females, eggs and larvae of *R. microplus*. Better results were observed when conidial oil-based suspension was used. In general, engorged females treated with 1×10^8 conidia ml^{-1} oil suspensions died before laying eggs, resulting in 97.6% of tick control. As far as we know, this is the first report of the effects of *L. lecanii* on *R. microplus* tick.

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1. Introduction

Rhipicephalus (*Boophilus*) *microplus*, known as the cattle tick, generally only parasitizes bovines; however, when the tick population reaches high levels of pasture infestations its parasitism may extend to other mammal species. Economic losses caused by ectoparasites in Brazil are estimated to be approximately 2.65 billion dollars a year, in which *R. microplus* is responsible for more than 75% of the losses (Grisi et al., 2002).

Parasite control, in general, is based on the use of chemical acaricides. Unfortunately, continual use of this method has many negative side effects, including the possibility of causing the development of chemical resistance in some ticks population, and food and environmental contamination if these products are improperly used. Public concerns about the environmental impact and safety of chemical applications are driving research into alternative, sustainable methods for tick control, including biological control (Chandler et al., 2000).

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Entomopathogenic fungi are important natural enemies of arthropods and can be used for biological control of ticks. Ticks, of all stages, were found naturally infected by several species of entomopathogenic fungi, including *Beauveria bassiana*, *Isaria fumosorosea* (=*Paecilomyces fumosoroseus*), *Isaria farinosa* (=*P. farinosus*) and *Lecanicillium lecanii* (=*Verticillium lecanii*) (Kalsbeek et al., 1995). One of the advantages of using entomopathogenic fungi to control ticks is that they penetrate through the integument (Madelin et al., 1967), meaning they do not have to be ingested. This type of penetration was also observed in the entomopathogenic fungus *Metarhizium anisopliae* on *R. microplus* cuticle by a histological study (Bittencourt et al., 1995). Later, conidial penetration of *M. anisopliae* and *B. bassiana* was further demonstrated on *R. microplus* by scanning electron microscopy (Bittencourt et al., 1999; Arruda et al., 2005; Campos et al., 2005).

The use of entomopathogenic fungi to control tick species has been tested worldwide with promising results (Samish and Rehacek, 1999; Chandler et al., 2000; Fernandes and Bittencourt, 2008). Isolation and characterization of new fungal species pathogenic to ticks should be continuously investigated and developed concurrently with the mycoacaricides for effective tick control. *B. bassiana* and *M. anisopliae* are the most commonly used fungi for microbial control of pest arthropods due to their large geographical distribution, wide host range, and capacity to cause enzootic and epizootic outbreaks (Alves, 1998).

The genus *Lecanicillium* was recently separated from *Verticillium* on the bases of molecular, ecological and morphological data. In the genus *Lecanicillium* there are several important entomopathogenic species: e.g., *Lecanicillium muscarium* and *Lecanicillium longisporum* which were used as biocontrol agents against aphids and whiteflies (Zare et al., 2000; Zare and Gams, 2001, 2004). Also, *L. lecanii* is a common pathogen that infects aphids and mealy bugs in tropical and neotropical regions, and was also reported to infect Coleoptera, Diptera, Hymenoptera, and Acari, such as *Ixodes ricinus*. Recently, *Lecanicillium psalliotae* was reported as pathogenic to *R. microplus*, especially to eggs and larvae, in laboratorial tests (Pirali-Kheirabadi et al., 2007); however, only a few studies have investigated the potential of *Lecanicillium* species to control ticks.

Formulation has been shown to be an important factor in achieving effective control using entomopathogenic fungi. Several studies have demonstrated that conidia suspended in oil are more effective in controlling arthropods populations than conidia suspended in a water solution (Prior et al., 1988; Kaaya and Hassan, 2000; Polar et al., 2005; Lopes et al., 2007). The proper formulation of a virulent entomopathogenic fungal isolate may improve the efficacy of biological control of ticks. Accordingly, the current study investigates the effectiveness of both water and oil-based conidial suspensions of *L. lecanii*, isolate CG 420, to control *R. microplus* engorged females, eggs and larvae. As far as we know, this is the first study that evaluates *L. lecanii* as a microbial agent for controlling *R. microplus*.

2. Materials and methods

2.1. Fungal isolate, cultivation and preparation of conidial suspension

L. lecanii, isolate CG 420, from Centro Nacional de Recursos Genéticos (CENARGEN, Embrapa, Brasília, DF, Brazil) was isolated from Brazilian soil (Aracaju, Sergipe State) in 1992. The fungus was cultivated on malt extract medium (MEM) at $25 \pm 1\,^{\circ}C$ and relative humidity (RH) $\geq 80\%$ for 15 days. Conidia were harvested from culture plates by scraping the medium surface with a plastic loop. A 30 ml suspension was prepared with harvested conidia in sterile aqueous 0.1% Tween 80 solution in a 50-ml-Falcon™ test tube. The conidial suspension was homogenized for 3 min using a vortex mixer. Conidia were also suspended in an oil solution of 15 ml sterile mineral oil (Vetec Química Fina Ltda., Rio de Janeiro, RJ, Brazil) with 84 ml sterile distilled water and 1 ml Tween 80. The oil solution was prepared according to Kaaya and Hassan (2000); however, peanut oil was substituted for mineral oil in equal quantities.

Conidial suspensions were quantified in hemacytometer and adjusted to 1×10^8 conidia ml^{-1}. The suspensions 1×10^7, 10^6 and 10^5 conidia ml^{-1} were prepared by serial dilutions. Conidia viability was determined by plating 100 μl of conidial suspensions on MEM and incubating at $25 \pm 1\,^{\circ}C$. Conidial germination was observed after 24 h (Alves, 1998).

2.2. Bioassays

Ticks were randomly assigned to one of seven treatment groups: five aqueous treatments containing 10 replicates in each group and two oil-based treatments with 30 replicates in each group. The aqueous treatments consisted of four conidial suspensions concentrated to 1×10^5, 10^6, 10^7 or 10^8 conidia ml^{-1} and a control group treated with 0.1% Tween 80 aqueous solution. The two oil-based treatments consisted of a conidial suspension at 1×10^8 conidia ml^{-1} and a control group (oil solution with no conidia). Bioassays were repeated twice on different days using different batches of conidia. In both bioassays, ticks were immersed in conidial suspensions or control solutions for 3 min.

2.2.1. Bioassay with engorged females

Engorged female ticks were obtained from calves that were artificially infested. Ticks were immersed in a 1% sodium hypochlorite solution for 5 min, rinsed with sterile distilled water and dried with sterile tissue paper for cuticle antisepsis. Engorged females between 0.2 and 0.28 g were selected for the bioassay, and separated into homogeneous groups (Sampaio, 2002).

After treatment, engorged females were attached to Petri plates, labeled and incubated at $27 \pm 1\,^{\circ}C$ and RH $\geq 80\%$. The eggs mass laid by each female was weighed daily and placed into individual test tubes. The eggs were then incubated at the same temperature and RH to allow larvae to hatch. The following parameters were evaluated:

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