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Entomopathogenic nematodes in insect cadaver formulations for the control of *Rhipicephalus microplus* (Acari: Ixodidae)

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ABSTRACT

This study evaluated the efficacy of four entomopathogenic nematode (EPN) strains in insect cadaver formulations against *Rhipicephalus microplus* and compared the efficacy of the most virulent EPNs applied in cadavers of *Galleria mellonella* and *Tenebrio molitor*. In the first experiment, infected *G. mellonela* larvae were used as the source of EPNs. Engorged females of *R. microplus* were placed in pots filled with soil and different numbers of *G. mellonella* larvae infected with one of four species of nematodes. All treatments with EPNs of the genus *Heterorhabditis* caused significant reduction ($p < 0.05$) in the egg mass weight and hatching percentage of larvae. The EPNs of the genus *Steinernema*, except for the group exposed to *Steinernema carpocapsae* ALL, whose source nematodes included six larvae of *G. mellonella*, caused a significant reduction ($p < 0.05$) in the egg mass weight produced per female. *Steinernema feltiae* SN applied with two, four, and six cadavers and *S. carpocapsae* ALL with two cadavers caused a reduction in hatching percentage of larvae of *R. microplus* ($p < 0.05$). The percentage of control was above 95% in all groups treated with *Heterorhabditis bacteriophora* HP88 and *Heterorhabditis indica* LPP1 and in the treatment with four larvae infected with *S. feltiae* SN. The second experiment followed the same methodology, using *G. mellonella* and *T. molitor* larvae infected by the two most virulent EPNs. *H. bacteriophora* HP88 and *H. indica* LPP1 in different formulations caused reduction in the egg mass weight and hatching percentage of larvae. The percentage of control were 82.4 and 84.9% for *H. bacteriophora* HP88 and *H. indica* LPP1, respectively, formulated in *T. molitor*, and reaching 99.9% in groups formulated with *G. mellonella*. The EPNs tested in insect cadaver formulation showed pathogenicity to engorged females of *R. microplus* and EPNs of the genus *Heterorhabditis* formulated in *G. mellonella* larvae were more effective.

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1. Introduction

For the past century, researchers have been trying to find alternatives to combat the cattle tick *Rhipicephalus microplus* (Canestrini, 1888), because of its extensive economic damage that this tick causes to milk and meat production systems in various regions of the world (Walker et al., 2003; Furlong et al., 2004; Martins et al., 2006; Furlong et al., 2007; Perreira, 2008). The application of chemical acaricides remains the main method of control; however, its systematic and often improper use has resulted in the selection of resistant ticks (Martins et al., 2006; Furlong et al., 2007; Labruna, 2008). In addition, there is a growing demand for new alternatives in pest control that use no chemicals, favoring preservation of the environment and production the residue-free foods (Samish, 2000; Dolinski, 2006).

Laboratory studies have shown that entomopathogenic nematodes (EPNs) represent a promising alternative for the control of *R. microplus* (Monteiro et al., 2010a, 2012; Silva et al., 2012). The biological method with the use of EPNs focusing the control on the non-parasitic phase of *R. microplus* can be effective, since engorged females at the time of oviposition seek environments with high moisture and protected from solar radiation, a characteristic that also favors the survival of EPNs. Previous simulation studies have shown that the application of different EPNs to the soil is effective against *Rhipicephalus annulatus* (Say, 1821) (Samish and Glazer, 2001; Alekseev et al., 2006).

One major factor affecting EPNs efficacy is the method of field application. Various formulations of EPNs are currently available, with the host cadaver formulation gaining importance (Dolinski, 2006). In this method, larvae of *Galleria mellonella* (Linnaeus, 1758) are infected with nematodes and released into the field. After a few days, the infective juveniles (IJs) begin to leave the cadavers to seek new hosts in the soil, thus controlling the targeted pest (Shapiro-Ilan et al., 2001). The cadaver provides protection against harmful biotic and abiotic factors, and the IJs released thus have greater energy reserves, greater ability to disperse and infect the host and greater longevity in the soil (Shapiro-Ilan et al., 2003). The implementation of this technology is simple, low in cost and can solve many of the current problems that prevent the extensive use of EPNs in pest control (Del Valle, 2008; Ansari et al., 2009). The success of this method has been reported in laboratory, greenhouse and field conditions (Del Valle et al., 2008; Ansari et al., 2009). In Brazil, this method has been used effectively for the control of *Conotrachelus psidii* (Marshall, 1922) in guava plantations in the municipality of Cachoeira de Macacu, Rio de Janeiro (Dolinski et al., 2012).

Larvae of *G. mellonella* have been used as hosts for the production of infected cadavers for EPNs (Del Valle et al., 2008) because these are highly susceptible insects and produce a large number of IJs (Dolinski, 2006). However, the production of *G. mellonella* larvae is expensive due to the cost of diet and the intensive handling they require (Costa et al., 2007). Another possibility is the use of larvae of *Tenebrio molitor* (Linnaeus, 1758) (Batista and Auad, 2010; Shapiro-Ilan et al., 2010), whose rearing is cheaper. However, this coleopteran produces fewer IJs per host compared to *G. mellonella* (Shapiro-Ilan et al., 2002; Molina et al., 2004).

Thus, this study aimed to evaluate the efficacy of *Heterorhabditis bacteriophora* (Poinar, 1876), strain HP88, *Heterorhabditis indica* (Poinar et al., 1982), strain LPP1, *Steinernema carpocapsae* (Weiser, 1955), strain All, and *Steinernema feltiae* (Filipjev, 1934) strain SN, in insect cadaver formulations against engorged females of *R. microplus* and also to compare the effectiveness of the more virulent EPNs produced in the cadavers of *G. mellonella* and *T. molitor*.

2. Materials and methods

The study was conducted at the Laboratory of Parasitology of the Embrapa Dairy Cattle Research Unit (Embrapa Gado de Leite), in Juiz de Fora, Minas Gerais, Brazil. This study used engorged females of *R. microplus* (Strain Porto Alegre "POA") obtained by artificial infestations in calves at the José Henrique Bruschi experimental farm of Embrapa, located in the municipality of Coronel Pacheco, Minas Gerais, Brazil (Registration in Ethics Committee on Animal Use of Embrapa 11/2013). The nematodes used were provided by the Laboratory of Entomology and Plant Pathology of Darcy Ribeiro Norte Fluminense Federal University and stored in the EPN bank at the Laboratory of Parasitology of Embrapa. The isolates (*H. bacteriophora* HP88, *H. indica* LPP1, *S. feltiae* SN, and *S. carpocapsae* ALL) were maintained by *in vivo* multiplication in *G. mellonella*.

2.1. Preparation of cadavers infected with EPNs

For the production of insect cadavers infected by EPNs, larvae of *G. mellonella* (groups of 10 larvae) with an average weight of 250 mg were placed in 9-cm diameter Petri dishes lined with two sheets of filter paper. This was followed by the addition of 2 mL of an aqueous solution containing nematodes (100 EPNs/mL) of each species being tested. The Petri dishes were then sealed with Parafilm and stored for three days in a chamber at 25 °C. Thereafter, the cadavers were transferred to 9-cm diameter Petri dishes lined with dry filter paper and incubated in the 25 °C for an additional four days (Del Valle, 2008).

The preparation of *T. molitor* cadavers infected with EPNs followed the same procedure as that described for *G. mellonela*, using larvae with average weight of 110 mg.

2.2. Experiment I

Plastic pots with a capacity of 300 mL were filled with 200 g of sterilized soil. Each container received 40 mL of distilled water to keep the soil at the limit of field capacity. Then cadavers (*G. mellonella*) with seven days of infection were buried in pots (day 0), and after six days (day 6), five engorged females were added. A control group was also created, consisting of engorged females placed in plastic pots free of nematodes. The females used in each treatment were divided into groups with