Parasitol Res (2012) 111:1473–1480
DOI 10.1007/s00436-012-2982-y

ORIGINAL PAPER

# Virulence of *Isaria* sp. and *Purpureocillium lilacinum* to *Rhipicephalus microplus* tick under laboratory conditions

**Isabele C. Angelo · Éverton K. K. Fernandes · Thiago C. Bahiense · Wendell M. S. Perinotto · Patricia S. Golo · Ana Paula R. Moraes · Vânia R. E. P. Bittencourt**

Received: 7 March 2012 / Accepted: 21 May 2012 / Published online: 19 June 2012


**Abstract** *Rhipicephalus microplus* (Canestrini) is an ectoparasite accountable for great economic losses. The use of entomopathogenic fungi to control arthropods has shown promising responses. The present study evaluated the virulence of *Isaria farinosa* (Holmsk.) Fr., *Isaria fumosorosea* (Wize) Brown and Smith, and *Purpureocillium lilacinum* (=*Paecilomyces lilacinus*) (Thom.) Samson to engorged females, eggs, and larvae of *R. microplus*. There were four treatment groups ($10^5$, $10^6$, $10^7$, and $10^8$ conidia $ml^{-1}$) and the control group (water and Tween 80, 0.1 %*v*/*v*). The treatment was based on immersion of the specimen in 1 ml of the suspension or control solution. The study observed changes in egg viability and larval mortality after treatment. The results showed that *I. farinosa*, *P. lilacinum*, and *I. fumosorosea* caused alterations in the biological parameters of *R. microplus* ticks. *I. fumosorosea* presented the greatest potential to control *R. microplus* engorged females in vitro, causing a 49 % decrease in nutritional index. All fungal isolates presented significant reduction in the egg production index. *I. farinosa* reduced the hatching percentage if the eggs were treated with the two highest conidial concentrations. All conidial concentrations of *I. fumosorosea* were able to reduce the hatching percentage significantly. All tested isolates showed pathogenicity toward unfed *R. microplus* larvae. As far as we know, this is the first study reporting the effect in vitro of *I. farinosa*, *I. fumosorosea*, and *P. lilacinum* to different developmental stages of *R. microplus* ticks.

I. C. Angelo · W. M. S. Perinotto · P. S. Golo · A. P. R. Moraes · V. R. E. P. Bittencourt (✉)
Animal Parasitology Department, Veterinary Institute, Universidade Federal Rural do Rio de Janeiro, Seropedica, Brazil
e-mail: vaniabit@ufrrj.br

É. K. K. Fernandes
Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goias, Brazil

T. C. Bahiense
Institute of Health Sciences, Universidade Federal da Bahia, Bahia, Brazil

## Introduction

Ticks are a considerable medical and veterinary public health concern due to direct damages caused by feeding and for their roles in transmitting well-known and emerging infectious agents (Bowman and Nuttall 2008). *Rhipicephalus microplus* Canestrini (1888), known as the cattle tick, is a hematophagous parasite widely spread in tropical and subtropical areas. The economic impact of major ectoparasites has been estimated in $2.6 billion per year in Brazil, including *R. microplus* that is responsible for $2 billion (Grisi et al. 2002). The exclusive use of chemical acaricides is becoming less advisable due to the increased costs, development of tick resistance to pesticides, and possible harm to the environment and human health (Barros and Evans 1989). Thus, it is important to find alternative control methods to optimize integrated pest control.

Entomopathogenic fungi are known for their specificity to target organisms under field conditions, especially during epidemics (Alves 1998). Many pathogenic fungi are known to be associated with ticks, including *Beauveria bassiana*, *Isaria fumosorosea*, *I. farinosa*, and *Lecanicillium* spp. Many studies have experimentally confirmed the pathogenicity of fungi, such as *B. bassiana* and *Metarhizium anisopliae*, to different tick developmental stages (Fernandes et al. 2006; Pirali-Kheirabadi et al. 2007; Fernandes and Bittencourt 2008; Ren et al. 2011). Fungal pesticides have been

proved to be efficient against a large variety of pests. Fungal pesticides are particularly indicated when the use of chemical pesticides is inappropriate and can cause the appearance of resistant tick populations or damage to the environment (Chandler et al. 2000).

Samish and Rehacek (1999) discussed the potential of biological control of ticks and concluded that biological pesticides based on entomopathogenic fungi are a promising alternative. However, it is necessary to discover and characterize more fungal species that are pathogenic to mites and ticks, along with the development of fungal acaricides. Recent advances in entomopathogenic fungal formulations have shown that it is possible to use fungal pesticides in environments where humidity was previously considered a limiting factor. Kaaya and Hassan (2000) demonstrated the efficiency of *M. anisopliae* or *B. bassiana* formulated in oil to control *Rhipicephalus decoloratus*, *Rhipicephalus appendiculatus*, and *Amblyomma variegatum*. This study reported that these fungi cause significantly high tick mortality and low egg viability of *R. decoloratus*, indicating that entomopathogenic fungi can be used as substitutes or integrated in tick control programs, mitigating the problem of chemical resistance. Moreover, Angelo et al. (2010) demonstrated the effect of an oil formulation of *Lecanicillium lecanii* against *R. microplus* eggs, larvae, and engorged females; *L. lecanii* formulated in oil was found to be more virulent in comparison to the aqueous formulation.

Studies about the ability of entomopathogenic fungi and their products to control agricultural pests have been conducted worldwide. However, biological control agents that affect ixodids and their pathogenic action are poorly understood. Further information about tick diseases, their susceptibility to entomopathogenic fungi and immune system behavior, are necessary to implement effective biological control methods (Monteiro et al. 2003). The present study aimed at evaluating the in vitro effects of the entomopathogenic fungi *I. farinosa*, *I. fumosorosea*, and *Purpureocillium lilacinum* to *R. microplus* eggs, larvae, and engorged females.

## Material and methods

### Entomopathogenic fungi

*P. lilacinum*, isolate CG 36; *I. farinosa*, isolate CG 198; and *I. fumosorosea*, isolate CG 202 from the Centro Nacional de Recursos Genéticos (CENARGEN, Embrapa, Brasília, DF, Brazil) were cultivated on potato dextrose agar medium at 25±1 °C and relative humidity (RH) ≥80 % for 15 days. Colonies of each isolate were evaluated as to colony diameter, appearance, and color of conidial masses and colony reverses. The micromorphology of each isolate was studied using the microculture technique between slide and coverslip (Rivalier and Seydel 1932). The dishes with cultures were kept under the same temperature and humidity conditions for 15 days. After that period, temporary slides were prepared and stained with Amman lactophenol with cotton blue (Hawksworth 1977).

**Table 1** Biological parameters of *R. microplus* engorged female treatment with aqueous conidial suspension of *Isaria farinosa, I. fumosorosea*, and *P. lilacinum*

| Treatment | Conidia $ml^{-1}$ | Engorged female mean initial weight (g) | NI | EPI | Percentage of tick control | Larvae hatchability (%) |
|---|---|---|---|---|---|---|
| Control | | 0.2392±0.02a | 69.65±8.9a | 58.21±6.0a | – | 97.8±3.5 |
| *Isaria farinosa* | $10^5$ | 0.2382±0.02a | 73.96±10.7a | 61.01±6.5a | 5.21 % | 88.3±20.8 |
| | $10^6$ | 0.2381±0.02a | 66.07±9.0a | 54.32±7.6bc | 15.17 % | 89.5±23.0 |
| | $10^7$ | 0.2390±0.02a | 69.54±10.6a | 56.17±7.6ab | 2.61 % | 98.9±1.7 |
| | $10^8$ | 0.2381±0.03a | 55.48±11.3b | 45.04±11.7c | 25.15 % | 95.4±5.9 |
| *Isaria fumosorosea* | $10^5$ | 0.2387±0.02a | 71.63±12.9a | 57.47±8.5ab | 33.05 % | 67.0±37.6b |
| | $10^6$ | 0.2397±0.02a | 60.30±22.2ab | 43.54±22.5c | 59.19 % | 51.2±38.6b |
| | $10^7$ | 0.2380±0.03a | 68.38±7.8b | 52.84±8.7bc | 46.16 % | 58.5±36.9b |
| | $10^8$ | 0.2365±0.03a | 59.02±23.0b | 48.95±18.9bc | 28.57 % | 83.6±31.3b |
| *Purpureocillium lilacinum* | $10^5$ | 0.2381±0.02a | 76.14±7.4a | 61.80±3.4a | −4.28 % | 96.1±5.9 |
| | $10^6$ | 0.2406±0.02a | 71.91±9.9a | 54.83±7.4b | 10.77 % | 91.8±20.0 |
| | $10^7$ | 0.2378±0.02a | 64.91±7.3a | 49.52±10.6b | 7.21 % | 96.6±3.2 |
| | $10^8$ | 0.2406±0.02a | 70.45±8.6a | 59.05±5.7ab | 13.50 % | 83.1±29.6 |

Experiments were conducted at 27±1 °C and RH ≥80 %. Means (±standard deviation) followed by the same letter in the same column do not differ statistically ($P \geq 0.05$). Means of ten replicates per bioassay. Bioassay was repeated twice

*NI* nutrient index (Bennett 1974), *EPI* egg production index (Drummond et al. 1971)

### Preparation of conidial suspensions

After the fungal isolates' growth, conidia were suspended in 30 ml of sterile distilled water plus 0.1 % Tween 80 (Luz et al. 1998). The conidial suspensions were measured and adjusted to $10^8$ conidia $ml^{-1}$, according to Alves (1998). Concentrations of $10^7$, $10^6$, and $10^5$ conidia $ml^{-1}$ of each isolate were prepared by serial dilutions.

### Bioassays

The bioassays for the different *R. microplus* development stages comprised four treatment groups ($10^5$, $10^6$, $10^7$, and $10^8$ conidia $ml^{-1}$) and one control group. The control group was immersed in water plus 0.1 % Tween 80 (with no conidia added), and the specimens from the four treatment groups were immersed in different conidial concentrations of each fungal isolate. The bioassays were performed twice on different days using different batches of conidia.

### Bioassay with engorged females

The methodology used in the treatment of engorged females was similar to that described by Angelo et al. (2010). Engorged females weighing between 0.23 and 0.25 g were selected for the bioassay and separated into homogeneous groups (Sampaio 2002). The following biological parameters were investigated: initial female weight, hatching percentage, residual weight of engorged females (engorged female weight 3 days after the end of oviposition), percentage of tick control (Drummond et al. 1971), egg production index (EPI), and nutrient index (NI) (Bennett 1974).

### Bioassay with eggs and unfed larvae

The methodology used in the treatment of eggs and unfed larvae was similar to that described by Angelo et al. (2010).

**Table 2** Hatching percentage of *R. microplus* larvae after treatment of eggs with conidial suspension of *I. farinosa*, *I. fumosorosea*, and *P. lilacinum*

| Treatment | *I. farinosa* | *I. fumosorosea* | *P. lilacinum* |
|---|---|---|---|
| Control | 98.8±2.4a | 98.8±2.4a | 98.8±2.4a |
| $10^5$ conidia $ml^{-1}$ | 97.7±0.8a | 64.8±2.1bc | 98.3±0.9a |
| $10^6$ conidia $ml^{-1}$ | 97.9±1.2a | 79.0±3.5b | 98.9±1.0a |
| $10^7$ conidia $ml^{-1}$ | 92.5±1.1b | 63.0±2.0c | 98.2±0.5a |
| $10^8$ conidia $ml^{-1}$ | 88.3±0.8b | 25.5±1.6d | 98.4±0.2a |

Experiments were conducted at 27±1 °C and RH ≥80 %. Means (±standard deviation) followed by the same letter in the same column do not differ statistically ($P \geq 0.05$). Means of ten replicates per bioassay. Bioassay was repeated twice

**Table 3** Mortality rate of *R. microplus* larvae treated with several concentrations of aqueous conidial suspensions of *I. farinosa*, *I. fumosorosea*, and *P. lilacinum*

| Treatments | Days after treatment | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *I. farinosa* | | | | *I. fumosorosea* | | | | *P. lilacinum* | | | |
| | 5 | 10 | 15 | 20 | 5 | 10 | 15 | 20 | 5 | 10 | 15 | 20 |
| Control | 0.0a | 0.0a | 0.0a | 1.0±0.3a | 0.0a | 0.0a | 0.0a | 1.0±0.2a | 0.0a | 0.0a | 0.0a | 1.0±0.5a |
| $10^5$ conidia $ml^{-1}$ | 0.0a | 0.0a | 2.0±1.2a | 10.0±2.3b | 0.0a | 0.0a | 1.0±0.7a | 6.0±2.6b | 0.0a | 0.0a | 0.5±0.2ab | 3.0±1.8ab |
| $10^6$ conidia $ml^{-1}$ | 0.0a | 6.0±2.5b | 16.5±2.4b | 26.0±3.8c | 0.0a | 0.0a | 1.5±0.9a | 4.5±1.8b | 0.0a | 0.0a | 1.0±0.5ab | 5.5±2.5b |
| $10^7$ conidia $ml^{-1}$ | 0.0a | 13.0±3.3c | 46.0±6.8c | 66.0±10.5d | 0.0a | 0.0a | 5.5±2.8b | 24.0±3.8c | 0.0a | 0.0a | 5.5±2.6b | 31.5±12.3c |
| $10^8$ conidia $ml^{-1}$ | 0.0a | 56.0±7.9d | 89.5±8.9d | 98.5±9.8e | 0.0a | 5.5b | 17.0±6.5c | 56.0±10.2d | 0.0a | 0.0a | 13.0±4.8c | 67.5±15.4d |

Mortality was observed at 5-day intervals for 20 days. Experiments were conducted at 27±1 °C and RH ≥80 %. Means (±standard deviation) followed by the same letter in the same column do not differ statistically ($P \leq 0.05$). Means of ten replicates per bioassay. Bioassay was repeated twice

**Table 4** The lethal concentrations of *I. farinosa*, *I. fumosorosea*, and *P. lilacinum* conidia required to cause either 50 or 90 % mortality ($LC_{50}$ and $LC_{90}$) of unfed larvae of *R. microplus* at different days after treatment

| Fungi | | Days after treatment | | | |
|---|---|---|---|---|---|
| | | 5 | 10 | 15 | 20 |
| *Isaria farinosa* | $LC_{50}$ | – | $8.98\times10^{8}$ conidia $ml^{-1}$ | $9.22\times10^{7}$ conidia $ml^{-1}$ | $3.11\times10^{7}$ conidia $ml^{-1}$ |
| | $LC_{90}$ | – | $2.57\times10^{10}$ conidia $ml^{-1}$ | $1.41\times10^{9}$ conidia $ml^{-1}$ | $4.87\times10^{8}$ conidia $ml^{-1}$ |
| *I. fumosorosea* | $LC_{50}$ | – | $9.05\times10^{14}$ conidia $ml^{-1}$ | $7.45\times10^{10}$ conidia $ml^{-1}$ | $8.55\times10^{8}$ conidia $ml^{-1}$ |
| | $LC_{90}$ | – | $2.83\times10^{19}$ conidia $ml^{-1}$ | $1.97\times10^{13}$ conidia $ml^{-1}$ | $5.52\times10^{10}$ conidia $ml^{-1}$ |
| *Purpureocillium lilacinum* | $LC_{50}$ | – | – | $2.63\times10^{11}$ conidia $ml^{-1}$ | $3.54\times10^{8}$ conidia $ml^{-1}$ |
| | $LC_{90}$ | – | – | $1.39\times10^{14}$ conidia $ml^{-1}$ | $8.14\times10^{9}$ conidia $ml^{-1}$ |

Bioassay was repeated twice

## Conidial viability

After preparation of conidial suspensions, a 10-μL aliquot of $10^{7}$ conidia-$ml^{-1}$ suspension of each isolate was transferred to Petri dishes containing potato dextrose agar (PDA) supplemented with 0.05 % chloramphenicol. The dishes were incubated at 25±1 °C and RH ≥80 %. After 24 h, the conidial viability was determined by direct optical

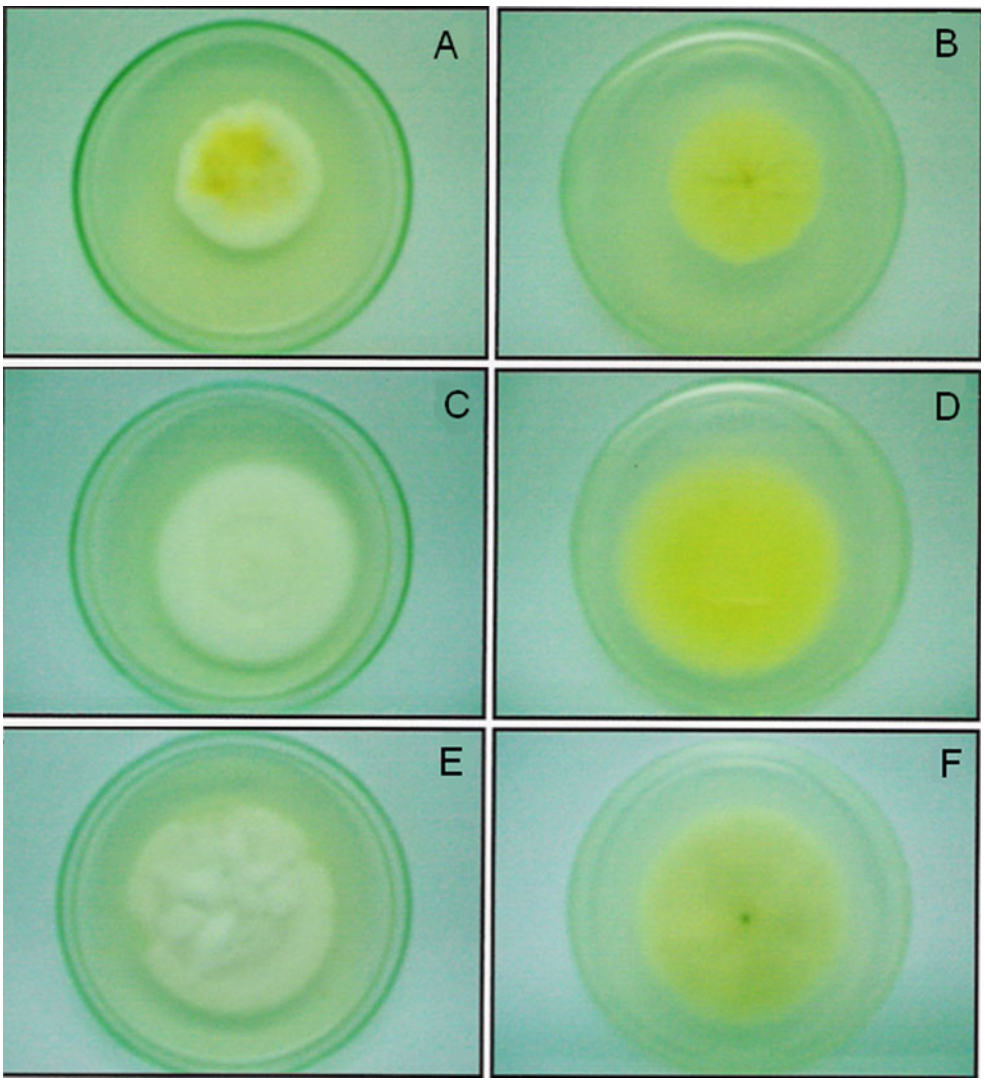


**Fig. 1** Surface and reverse colony of *I. farinosa* (**a**, **b**), *I. fumosorosea* (**c**, **d**), and *P. lilacinum* (**e**, **f**) used in bioassays with *R. microplus*. Fungal isolates were identified according to Samson (1974). The fungi, obtained from the CENARGEN collection (Empresa Brasileira de Pesquisa Agropecuária–EMBRAPA, Recursos Genéticos e Biotecnologia), were inoculated on a 23-mL PDA medium in a Petri plate (95×15 mm) and held in the dark at 25 °C for 14 days

microscopic observation at ×400 magnification. Two-hundred conidia were counted, and the number of germinated conidia was divided by 200 and multiplied by 100 to reach the viability percentage (Alves 1998).

### Re-isolation of entomopathogenic fungi

Eggs, larvae, and dead engorged females from all treatment groups were incubated at 25±1 °C and RH ≥80 % to allow fungal growth and conidiogenesis. After 14 days, the fungi were spread on dishes with PDA supplemented with 0.05 % chloramphenicol to evaluate the macro- and micromorphology, according to Samson (1974).

### Statistical analysis

The parametric data (pre-oviposition period, oviposition period, egg incubation period, and hatching period) were assessed using analysis of variance followed by the Student–Newman–Keuls test. Nonparametric data (nutrient index, egg production index, and hatching percentage) were assessed by the Kruskal–Wallis test followed by Student's *t* test. *P* values less than 0.05 were considered significant. The lethal concentrations (LC), $LC_{50}$ and $LC_{90}$, were assessed using the method of Probit analysis (Finney 1971) generated by Probit or Logit Analysis, POLO-PC (LeOra Software 1987).

## Results

### Conidial viability

The conidial suspensions of *I. farinosa*, *I. fumosorosea*, and *P. lilacinum* presented viability higher than 98 %, indicating that conidia were able to cause infection to all the tick developmental stages investigated.

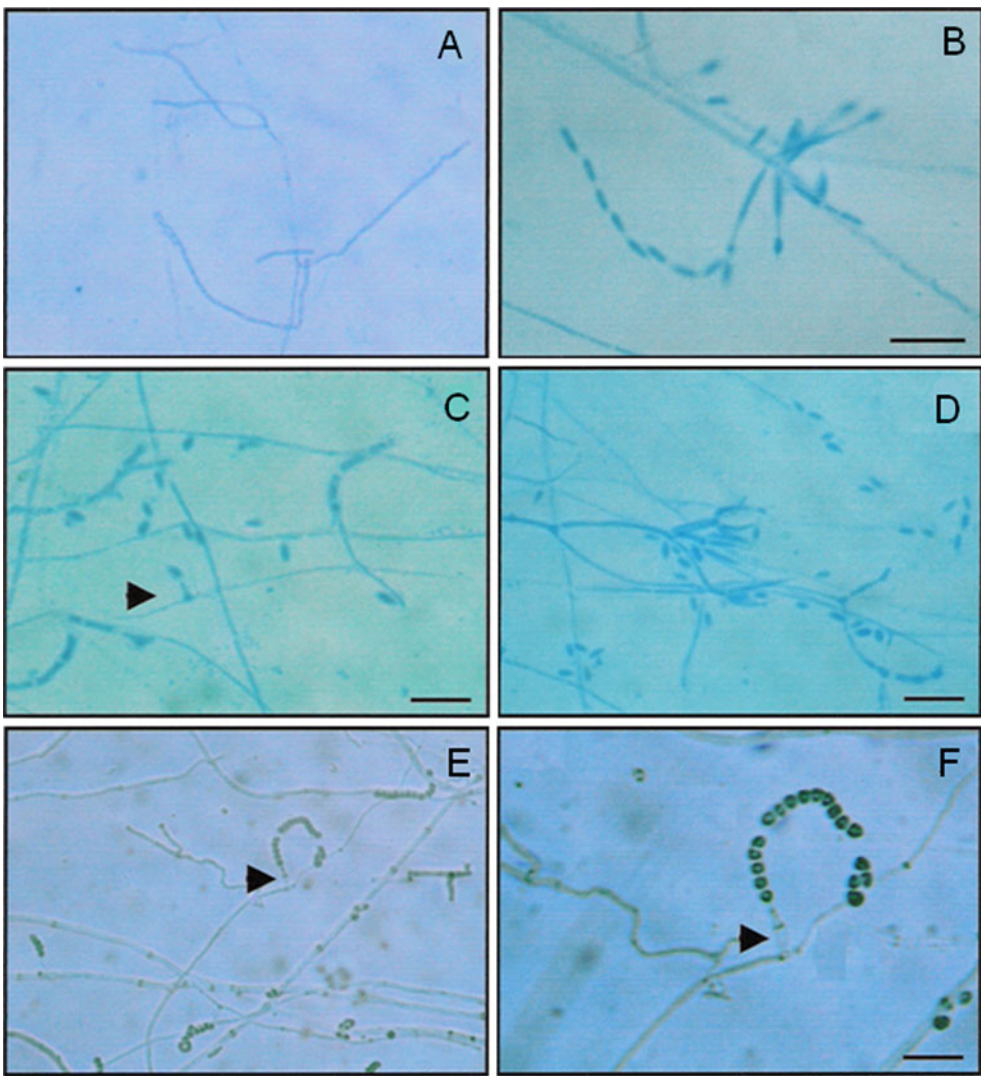


**Fig. 2** Micromorphology of *I. farinosa*, *I. fumosorosea*, and *P. lilacinum* used in bioassays with *R. microplus*. Fungal isolates were observed under optical microscopy at ×400 magnification, and the fungi were identified according to Samson (1974). **a** *I. farinosa*: solitary conidiogenous cells and conidia in imbricate chains at ×400 magnification; **b** *I. farinosa*: flask-shaped conidiogenous cells; **c** *I. fumosorosea*: globose conidiogenous cells; **d** *I. fumosorosea*: conidiophore; **e** *P. lilacinum* at ×400 magnification; **f** *P. lilacinum*: flask-shaped conidiogenous cells at ×1,000 magnification. *Bars*=10 μm

### Bioassay with engorged females

The NI of the engorged females in the control and treated groups is shown in Table 1. The *I. fumosorosea* isolate at the concentrations of $10^7$ and $10^8$ conidia $ml^{-1}$ caused significant reduction in the NI ($P<0.05$). The percentages were 59.02 % in the group treated with $10^8$ conidia $ml^{-1}$ and 69.65 % in the control group. Similar results were observed with the *I. farinosa* isolate, but only at the highest conidial concentration ($P<0.05$). Conversely, *P. lilacinum*, isolate CG 36, was not able to significantly reduce this parameter ($P>0.05$).

The engorged females in the control group showed EPI values similar to those found by Davey et al. (1980), which means that the females used in this study had similar parameters to those already described in the literature. All fungal isolates caused significant reduction ($P<0.05$) in the EPI of treated engorged females in comparison with ticks from the control group (Table 1).

There was no significant reduction ($P<0.05$) in the hatching percentage of larvae obtained from eggs from engorged females treated with different conidial concentrations of *I. farinosa* and *P. lilacinum*. *I. fumosorosea* was the only isolate which reduced the hatching percentage ($P<0.05$) when the concentrations of $10^6$ and $10^7$ conidia $ml^{-1}$ were used to treat the engorged females. Accordingly, *I. fumosorosea* was more efficient in controlling *R. microplus* causing a control percentage of 59.19 %, while it was 25.15 % for *I. farinosa* and 13.5 % for *P. lilacinum* (Table 1).

### Egg bioassay

*I. farinosa* reduced the hatching percentage of larvae when eggs were treated with the two highest conidial concentrations ($10^7$ or $10^8$ conidia $ml^{-1}$) ($P<0.05$), whereas *P. lilacinum* did not reduce significantly ($P>0.05$) the hatching percentage of larvae (Table 2). All conidial concentrations of *I. fumosorosea* were able to reduce the hatching percentage significantly ($P<0.05$) (Table 2).

### Unfed larvae bioassay

*Isaria* isolates caused *R. microplus* larval mortality at day 10 after treatment ($P<0.05$). The mean percentage mortality varied from 2.0 to 98.5 % for treatments with *I. farinosa* and 1.0 to 56 % for *I. fumosorosea* (Table 3). *P. lilacinum* showed an effect against larvae at day 15 after treatment, and 13.0 % mortality occurred among the larvae treated with the highest conidial concentration tested; at day 20 after treatment, this percentage ranged from 3.0 to 67.5 % among the treatment groups. For all isolates, the percentage mortality was higher as the conidial concentration increased. The lethal concentrations ($LC_{50}$ and $LC_{90}$) of the different conidial suspensions tested are shown in Table 4.

### Entomopathogenic fungi re-isolation

Egg and larval samples showed fungal growth after incubation under controlled temperature and humidity, but the fungal colonization on the females' cuticle was only observed in the groups treated with *I. farinosa*. Fungal colonies were evaluated for their macro- and micromorphological characteristics and identified as the same fungal species used in bioassays with *R. microplus*, confirming that the entomopathogenic fungi were responsible for the tick infection (Figs. 1 and 2). Samples from the control group were kept under the same conditions and showed no development of fungal colonies (Fig. 3).

## Discussion

In general, entomopathogenic fungi have been shown to be pathogenic to ticks (Samish and Rehacek 1999; Chandler et al. 2000; Gindin et al. 2002; Samish et al. 2004; Fernandes and Bittencourt 2008; Angelo et al. 2010). In the current study, the significant reduction in the NI of the engorged females experimentally infected with *I. farinosa* and *I. fumosorosea* demonstrated that these fungi interfere in the reproductive capacity of *R. microplus*. Bittencourt et al. (1997) observed that the NI of *R. microplus* engorged females decreased as the *B. bassiana* conidial concentration increased. In the present study, this result was observed only with the two highest conidial concentrations of *I. fumosorosea* and *I. farinosa*.

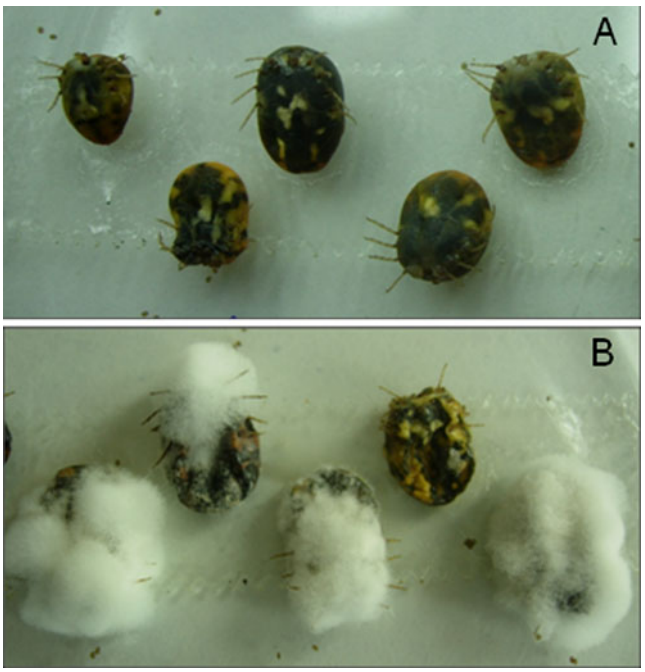


**Fig. 3** Engorged females of *R. microplus* at day 10 after treatment. **a** Tick from the control group (not infected) and **b** tick treated with *I. fumosorosea* aqueous conidial suspension at $1\times10^8$ conidia $ml^{-1}$

All isolates showed significant reduction in the EPI compared with the control group (see Table 1). The EPI reflects the relationship between the nutrients ingested by the female during the parasitic phase and the energy used to produce the egg mass. The engorged females in the control group and in the treated groups showed no significant difference in their initial weight (see Table 1); then, the amount of blood ingested by each female was similar in both treatment and control groups. However, the females treated with the fungal isolates were unable to make appropriate conversion of nutrients into eggs (EPI was significantly reduced in comparison to the control groups), indicating that the subsequent generation of ticks will be potentially reduced and the tick population controlled. Gindin et al. (2001) showed a reduction in fertility of *Rhipicephalus annulatus* (=*Boophilus annulatus*) engorged females treated with *I. fumosorosea*. This study assessed the effectiveness of two *I. fumosorosea* strains to *R. annulatus*, *Rhipicephalus sanguineus*, and *Hyalomma excavatum* and observed reduction of the egg mass laid by infected engorged females.

*I. fumosorosea* showed the highest control percentage in bioassays with engorged females; however, the highest control percentage was not achieved with the highest conidial concentration. This fact was possibly due to the competition among the conidia at the beginning of the penetration process through the cuticle. Moreover, the $10^7$ conidia-ml$^{-1}$ concentration also was effective in reducing the hatching percentage in the bioassay with engorged females and in the bioassay with eggs ($P<0.05$), indicating that the entomopathogenic fungus *I. fumosorosea* can be considered as a promising agent to control *R. microplus*. Gindin et al. (2001) reported that *B. bassiana* and *I. fumosorosea* did not normally emerge from the tick cuticle but through the natural openings instead. In the current study, we observed cuticle fungal growth of *I. fumosorosea* on dead females at day 10 after treatment (see Fig. 3).

The three entomopathogenic fungi showed pathogenicity to unfed larvae of *R. microplus*. Samish et al. (2001) observed that two *I. fumosorosea* isolates caused less than 10 % mortality of *R. sanguineus* larvae, 7 days after treatment. These data are similar to those found in this study, in which *I. fumosorosea* caused only 5.5 % mortality of *R. microplus* larvae at day 10 after treatment. This fungus has probably little pathogenic potential for tick larvae, requiring a longer period to cause high mortality. Polar et al. (2005) observed that *I. farinosa* was not pathogenic to any of the *R. microplus* developmental stages tested. Conversely, the present study showed the pathogenic effect of *I. farinosa* to *R. microplus* larvae at day 10 after treatment; at day 20 after treatment, the mean mortality was close to 100 %. This difference can be accredited not only to the fungal isolate tested, but also to the different methods employed.

Even though the effectiveness of entomopathogenic fungi against ticks has been confirmed by several authors, the virulence of these fungi can vary considerably depending on the isolate tested. In this respect, Fernandes et al. (2006) studied the genetic variability of 50 *B. bassiana* isolates from different geographical regions and observed a variation of 3 to 100 % mortality of artificially infected *R. microplus* unfed larvae.

The $LC_{50}$ value of *I. farinosa*, isolate CG 198, obtained at day 15 after treatment was lower than those reported by Reis et al. (2001) when they studied the effect of three *M. anisopliae* isolates to unfed nymphs of *Amblyomma cajennense*. All isolates used in this study showed $LC_{90}$ values lower than those reported by Reis et al. (2001); however, *I. farinosa* has the potential to control *R. microplus*, once the conidial concentrations corresponding to the $LC_{50}$ and $LC_{90}$ are easily prepared.

*I. farinosa*, *I. fumosorosea*, and *P. lilacinum* demonstrated pathogenic effects to *R. microplus*, but the virulence varied according to the developmental stage of the tick. *I. fumosorosea* presented the highest potential to control *R. microplus* engorged females. All tested isolates showed pathogenicity toward *R. microplus* unfed larvae. Other isolates of *I. farinosa*, *I. fumosorosea*, and *P. lilacinum* should be tested against ticks once different levels of virulence have been detected among isolates of the same species of entomopathogenic fungus to many arthropod species, including ticks (Alves 1998; Lomer et al. 2001; Fernandes et al. 2006, 2009, 2010; Pirali-Kheirabadi et al. 2007; Ren et al. 2011). As far as we know, this is the first report of virulence of *I. farinosa*, *I. fumosorosea*, and *P. lilacinum* to *R. microplus* ticks.

**Acknowledgments** We are grateful to the National Center for Genetic Resources (CENARGEN)/Brazilian Agricultural Research Corporation (EMBRAPA) for providing the fungal isolate used in this study. This research was supported by grants from the National Council for Scientific and Technological Development (CNPq) and the Rio de Janeiro State Research Foundation (FAPERJ). We also thank the Office to Improve University Research (CAPES) for providing the M.Sc. and Ph.D. scholarship to I.C.A., É.K.K. F., T.C.B., W.M.S.P., P.S.G., and A.P.R.M. V.R.E.P. Bittencourt is CNPq researcher (1B).

## References

Alves SB (1998) Controle microbiano de insetos, 2nd edn. FEALQ, Piracicaba

Angelo IC, Fernandes EK, Bahiense TC, Perinotto WMS, Moraes APR, Terra ALM, Bittencourt VREP (2010) Efficiency of *Lecanicillium lecanii* to control the tick *Rhipicephalus microplus*. Vet Parasitol 172:317–322

Barros TAM, Evans DE (1989) Ação de gramíneas forrageiras em larvas infectantes do carrapato dos bovinos *Boophilus microplus*. Pesquisa Vet Brasil 9:17–21

Bennett GF (1974) Ovoposition of *Boophilus microplus* (CANESTRINI) (ACARIDA: IXODIDAE) I. Influence of tick size on egg production. Acarologia 16:52–61

Bittencourt VREP, Peralva SLFS, Souza EJ, Mascarenhas AG, Alves SB (1997) Ação de dois isolados do fungo entomogênico *Beauveria bassiana* sobre algumas características biológicas de fêmeas

ingurgitadas de *Boophilus microplus* em laboratório. Rev Univ Rural Ser Cienc Vida 19:65–71

Bowman AS, Nuttall P (2008) Ticks—biology, disease and control. Cambridge University Press, UK

Chandler D, Davidson G, Pell JK, Ball BV, Shaw K, Sunderland KD (2000) Fungal biocontrol of acari. Biocontrol Sci Techn 10:357–384

Davey RB, Garza JG, Thompson GD, Drummond RO (1980) Ovipositional biology of the southern cattle tick, *Boophilus microplus* (Acari: Ixodidae) in the laboratory. J Med Entomol 17:117–121

Drummond RO, Gladney WJ, Whetstone TM, Ernst SE (1971) Laboratory testing of insecticides for control of the winter tick. J Econ Entomol 64:686–688

Fernandes EKK, Bittencourt VREP (2008) Entomopathogenic fungi against South American tick species. Exp Appl Acarol 46:71–93

Fernandes EKK, Costa GL, Moraes AML, Zahner V, Bittencourt VREP (2006) Study on morphology, pathogenicity, and genetic variability of *Beauveria bassiana* isolates obtained from *Boophilus microplus* tick. Parasitol Res 98:324–332

Fernandes EKK, Moraes AML, Pacheco RS, Rangel DEN, Miller MP, Bittencourt VREP, Roberts DW (2009) Genetic diversity among Brazilian isolates of *Beauveria bassiana*: comparisons with non-Brazilian isolates and other *Beauveria* species. J Appl Microbiol 107:760–774

Fernandes EKK, Keyser CA, Chong JP, Rangel DEN, Miller MP, Roberts DW (2010) Characterization of *Metarhizium* species and varieties based on molecular analysis, heat tolerance and cold activity. J Appl Microbiol 108:115–128

Finney DS (1971) Probit analysis, 3rd edn. Cambridge University Press, Cambridge

Gindin G, Samish M, Alekseev E, Glazer I (2001) The susceptibility of *Boophilus annulatus* (Ixodidae) ticks to entomopathogenic fungi. Biocontrol Sci Techn 11:111–118

Gindin G, Samish M, Zangi G, Mishoutchenko A, Glazer I (2002) The susceptibility of different species and stages of ticks to entomopathogenic fungi. Exp Appl Acarol 28:283–288

Grisi L, Massard CL, Borja GEM, Pereira JB (2002) Impacto econômico das principais ectoparasitoses em bovinos no Brasil. Hora Vet 21:23–28

Hawksworth DL (1977) Micologist's handbook, 2nd edn. CAB Press, Kew

Kaaya GP, Hassan S (2000) Entomogenous fungi as promising biopesticides for tick control. Exp Appl Acarol 24:913–926

Lomer CJ, Bateman RP, Johnson DL, Langewald J, Thomas M (2001) Biological control of locusts and grasshoppers. Annu Rev Entomol 46:667–702

Luz C, Tigano MS, Silva IG, Cordeiro CMT, Aljanabi SM (1998) Selection of *Beauveria bassiana* and *Metarhizium anisopliae* isolates to control *Triatoma infestans*. Mem Inst Oswaldo Cruz 93:839–846

Monteiro SG, Bahiense TC, Bittencourt VREP (2003) Ação do fungo *Beauveria bassiana* (Balsamo) Vuillemin, 1912 sobre a fase parasitária do carrapato *Anocentor nitens* (Neumann, 1897) Schulze, 1937 (Acari: Ixodidae). Cienc Rural 33:559–563

Pirali-Kheirabadi K, Haddadzadeh H, Razzaghi-Abyaneh M, Bokaie S, Zare R, Ghazavi M, Shams-Ghahfarokhi M (2007) Biological control of *Rhipicephalus* (*Boophilus*) *annulatus* by different strains of *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium psalliotae* fungi. Parasitol Res 100:1297–1302

Polar P, Kairo MTK, Peterkin D, Moore D, Pegram R, John S (2005) Assessment of fungal isolates for development of a myco-acaricide for cattle tick control. Vector-Borne Zoonot 5:276–284

Reis RCS, Melo DR, Souza EJ, Bittencourt VREP (2001) Ação in vitro dos fungos *Beauveria bassiana* (Bals.) Vuill e *Metarhizium anisopliae* (Metsch.) Sorok sobre ninfas e adultos de *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae). Arq Bras Med Vet Zoo 53:544–547

Ren Q, Sun M, Guan G, Li Y, Liu Z, Liu A, Ma M, Niu Q, Liu J, Yin H, Luo J (2011) Biological control of engorged female *Haemaphysalis qinghaiensis* (Acari: Ixodidae) ticks with different Chinese isolates of *Beauveria bassiana*. Parasitol Res 109:1059–1064

Rivalier E, Seydel S (1932) Nouveau procede de culture sur lames gélosés appliqué a l'étude microscopique de champignos deteignes. Ann Parasitol 10:444–452

Samish M, Rehacek J (1999) Pathogens and predators of ticks and their potential in biological control. Annu Rev Entomol 44:159–182

Samish M, Gindin G, Alekseev E, Glazer I (2001) Pathogenicity of entomopathogenic fungi to different developmental stages of *Rhipicephalus sanguineus* (Acari: Ixodidae). J Parasitol 87:1355–1359

Samish M, Ginsberg H, Glazer I (2004) Biological control of ticks. Parasitology 129:389–403

Sampaio IBM (2002) Estatística Aplicada à Experimentação Animal, 2nd edn. FEPMVZ, Belo Horizonte

Samson RA (1974) *Paecilomyces* and some allied Hyphomycetes. Stud Mycol 6:120