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Effect of isolate IBCB 130 of the fungus *Purpureocillium lilacinum* on the tick *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae)

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ABSTRACT

The importance of in-vitro tests using larvae and adults of the tick *Rhipicephalus (Boophilus) microplus* in investigating new inputs for its biological control serves as a basis for continuation of field experiments. This article presents in-vitro efficacy data on the fungus *Purpureocillium lilacinum* (IBCB 130) tested using larvae and engorged females of the tick *R. microplus* according to modified techniques of SHAW (1966) and MONTEIRO (2014). The efficacy results from the tests on engorged females ranged from 99.6 at the highest concentration used ($2x10^9$ conidia/mL) to 51% at the lowest concentration ($1x10^7$ conidia/mL). The 50, 90 and 99% lethal concentrations found in the tests on larvae were, respectively, $5.1x10^8$, $5.2x10^9$, and $3.3x10^{11}$ conidia/mL. The results from this investigation have enabled field experiments to control the tick *R. microplus* using the IBCB 130 isolate of the fungus *P. lilacinum*.

Keywords: biological control; entomopathogenic fungus; bioassay.

INTRODUCTION

The tropical climate of Brazil favors the presence of the cattle tick *Rhipicephalus (Boophilus) microplus* throughout the year, especially in the country's southeastern region. This parasite causes damage to Brazilian livestock through decreasing milk and meat yields and losses relating to leather production and the spread of pathogens of the genera *Babesia* and *Anaplasma*. The total economic losses are approximately US$ 3.24 billion per year in Brazil (MELO et al., 2006; GRISI et al., 2014).

Rhipicephalus microplus is traditionally controlled through the use of chemical products, often without any technical guidance. This lack of guidance accelerates the selection of resistant strains, and impacts food safety through the presence of chemical residues in products of animal origin (ANDREOTTI et al., 2019).

Integrated management is the proper tool for ensuring that infestation of cattle by ticks does not cause damage to the animals themselves or to public health and the environment. Application of fungi to pastures concomitantly with chemical treatment of animals favors the reduction of the infestation levels of this ectoparasite. According to Santos et al. (2022), applications of the fungus *Metarhizium anisopliae* (IBCB 425) to pastures showed tick control efficacies of 36 to 48%.

The fungus *Purpureocillium lilacinum* is characterized by a filamentous appearance with rough walls and chains of conidia with viscous heads. This species develops in a variety of environments due to its pH tolerance and its wide

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temperature range (between 8 and 38°C). In addition, it is commonly found in soil and decomposing organic matter (PERDOMO et al., 2012, SRILAKSHMI et al., 2017).

In agriculture, this fungus has been used for controlling phytoparasitic nematodes (root-knot nematodes, genus *Meloidogyne*), as an insecticide (cotton aphids, *Aphis gossypii*) and as an acaricide (mites, *Tetranychus urticae*). Records of biopesticides based on *P. lilacinum* have already been reported in China, United States of America and Europe (LUANGSA-ARD et al., 2011; LOPEZ et al., 2014; WANG et al., 2016; XIE et al., 2016; CHEN; HU, 2022; LIU et al., 2022).

In Brazil, several research projects have been developing experiments on biological products (fungi, nematodes, and bacteria) with the aim of reducing the use of chemicals. Research on species and isolates of different fungi is necessary in order to offer companies and on-farm producers of biomaterials new possibilities for biological control. This article presents in-vitro efficacy data on the fungus *P. lilacinum* (IBCB 130) tested with the tick *R. microplus*.

MATERIAL AND METHODS

Tick samples

Engorged females of *R. microplus* were collected from cattle kept at the Pindamonhangaba Research and Development Unit (latitude: 22°55'26"S; longitude: 45°27'42"W; altitude: 557 m).

Obtaining fungal isolates

The fungus *P. lilacinum* (IBCB 130) was made available by the Laboratory Reference Unit for Biological Control of the Advanced Research Center on Plant Protection and Animal Health, at the Biological Institute, in the municipality of Campinas, state of São Paulo, Brazil.

Preparation of fungal suspension

Fungal suspensions of *P. lilacinum* were prepared at the following concentrations: $2x10^9$; $1x10^9$; $5x10^8$; $1x10^8$; $5x10^7$; and $1x10^7$ conidia/mL. These concentrations were prepared in aqueous solution with 0.01% of tween 80, and the same solution was used for the control group.

Bioassays

Engorged females of *Rhipicephalus microplus*

The biological assay was based on the modified technique (MONTEIRO, 2014). The engorged females were selected, weighed on a precision scale and placed in Petri dishes (60 × 15 mm) lined with two sheets of filter paper. Next, 1 mL of the aqueous fungal suspensions was added to the plates with homogeneous distribution. Then, the plates were closed and sealed with parafilm and kept in a heated chamber at 28°C and relative humidity of 80% for three days. After these three days, the filter paper and parafilm were removed, leaving only the Petri dish covers, and these plates were then returned to the heated chamber for another 15 days. After this period, the eggs were weighed and placed in test tubes duly labeled and closed using cotton wool dampened with water. The test was performed with 36 replicates for each concentration and control. The percentage inhibition of oviposition and efficacy were calculated as described by Drummond et al. (1973).

Immersion test on *Rhipicephalus microplus* larvae

Engorged *R. microplus* females were separated for oviposition in Petri dishes identified with the collection site and date of arrival at the laboratory. After a 15-day period, the eggs were placed in labeled glass jars capped with damp cotton wool, for the larvae to hatch in.

The immersion test on larvae aged 14 to 21 days was performed in accordance with the adapted SHAW'S technique (1966). One mL of fungal suspension was placed in a disposable Petri dish containing filter paper, at the concentration specified on the filter paper. Then, with the aid of a brush, approximately 100 larvae were added to each dish. Another piece of filter paper was then placed on top of the larvae, and 1 mL of the fungal suspension was applied at the same concentration. After 10 minutes, the filter papers with the larvae were removed from the dish. After removal of excess suspension, one of these two pieces of filter paper was inserted, together with the larvae, into a labelled test tube that was closed with dampened cotton wool. The tubes were stored in a biochemical oxygen demand heated chamber at the temperature of 28°C and 80% relative humidity. The same procedure was performed for the control group. The mortality rates on the fifth, 10th and 15th day after immersion of the larvae were evaluated with the aid of an LEICA EZ4W magnifying glass. The lethal concentrations LC_{50}, LC_{90} and LC_{99} were calculated from the larval mortality data using the Polo Plus LeOra software, version 1.0 (LEORA, 2017).

RESULTS

The data on the mean percentage inhibition of oviposition and efficacy of each concentration of the fungus *P. lilacinum* tested on the tick *R. microplus* are presented in Table 1. In general, the standard deviation values showed variations in the efficacy data, except for the results obtained at the highest concentration (2.10^9 conidia/mL). The highest mean percentage inhibition of oviposition was 48% at the highest concentration of the fungal suspension. However, in relation to efficacy, the mean percentage ranged from 99.6% at the highest concentration to 51% at the lowest one (1x10^7 conidia/mL).

Table 1. Mean percentage inhibition of oviposition and efficacy of engorged females of the tick *Rhipicephalus microplus* tested at different concentrations of the fungus *Purpureocillium lilacinum*.

Concentrations*	Posture inhibition and standard deviation	Effectiveness and standard deviation
2x10^9	48% (17.4)	99.6% (0.9)
10^9	25.8% (37.7)	85.2% (24.4)
5x10^8	31.5% (35.7)	80% (22.6)
10^8	29.4% (21.6)	73.3% (30.2)
5x10^7	26.4% (24.7)	61% (33.6)
10^7	21.3% (16.6)	51% (26.6)

*Conidia concentration/mL.
Source: elaborated by the authors.

There was no larval mortality on the fifth or 10th day of evaluation at any of the concentrations. However, on the 15th day, larval mortality was observed, and the 50, 90 and 99% lethal concentrations were 5.1 × 10^8, 5.2 × 10^9, and 3.3 × 10^{11} conidia/mL, respectively (Table 2 and Fig. 1).

Table 2. 50% lethal concentration (LC_{50}), 90% lethal concentration (LC_{90}) and 99% lethal concentration (LC_{99}) of the fungus *Purpureocillium lilacinum* tested on larvae of the tick *Rhipicephalus microplus*.

Number of larvae	50% lethal concentration	Fiducial limit	90% lethal concentration	Fiducial limit	99% lethal concentration	Fiducial limit
10,370	5.1.10^8	4.1.10^8–6.5.10^8	5.2.10^9	3.7.10^9–7.7.10^9	3.3.10^{11}	1.9.10^{11}–7.6.10^{11}

*Conidia concentration/mL. Source: Elaborated by the authors.

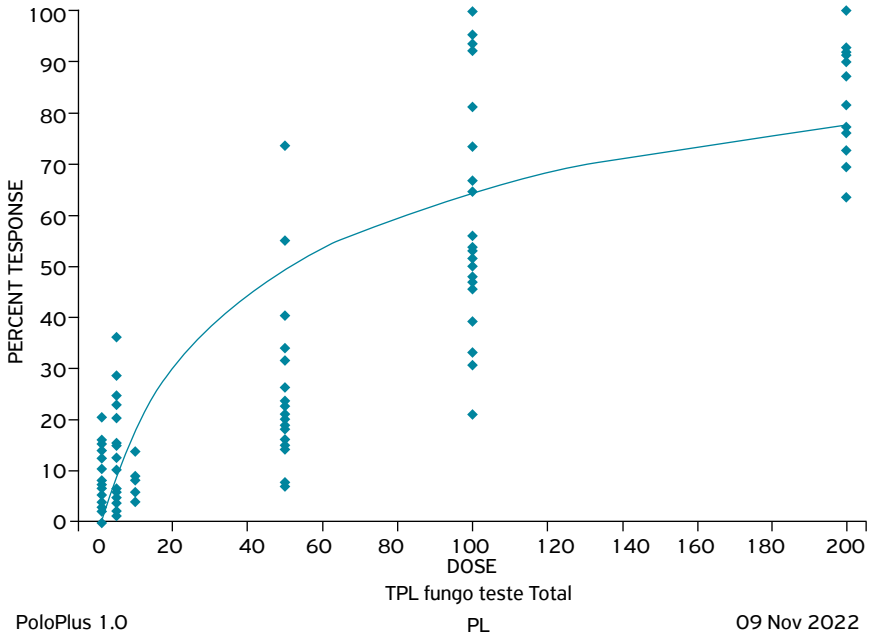

Figure 1. Mortality of *Rhipicephalus microplus* tick larvae tested using the fungus *Purpureocillium lilacinum*.
Source: Elaborated by the authors.

DISCUSSION

The metabolites produced by *P. lilacinum*, such as leucinostatins, have bactericidal, fungicidal, and antitumor actions. This fungus has enzymes that degrade chitin and the protein components of the epidermis of its hosts (nematodes, insects, and mites). This process can be optimized through the action of the specific 6-oxychitin product of chitin C-6 oxidation (CHEN; HU, 2022).

In livestock-rearing, the fungus *P. lilacinum* has been tested as both an endoparasiticide and an ectoparasiticide. It was seen to have efficient ovicidal action in the forms of raw and filtered macerated extract of *P. lilacinum*, against trichostrongylid endoparasites of sheep (BAPTISTA et al., 2020).

The fungal efficacy data from the present experiment were 61% at the lowest concentration (10^7 conidia/mL) and 99% at the highest concentration (2.10^9 conidia/mL). These data differed from what was previously reported by Angelo et al. (2012), who tested the isolate CG 36 of *P. lilacinum* at concentrations of 10^6, 10^7 and 10^8 conidia/mL, which yielded efficacies of 10.77, 7.21 and 13.5%, respectively. Furthermore, in comparing the results obtained by Angelo et al. (2012), there were differences in terms of fungal efficacy against engorged females and larvae, such as that their isolate only showed significant efficacy against the larvae of this tick.

It was observed that the efficacy of the action of the isolate IBCB 130 of *P. lilacinum* was obtained mainly through its action on the hatching of the larvae, since the percentage inhibition of oviposition among the treated females was low.

The larval mortality shown in the present study beyond the 15th day of treatment was also reported by Angelo et al. (2012). However, the 50 and 90% lethal concentrations found by those authors were higher than what we found in this study. The possible explanations for such differences may be related to the methodology used or to the higher virulence of the IBCB isolate, compared with the CG 36 isolate of *P. lilacinum*.

Investigation of new materials for biological control of pests in livestock-rearing is important because this tends to protect the quality of chemical products through reduced use of these products. It also provided new possibilities for producers, given the variety of species and isolates available for combating pests. In addition to the fungi *Metarhizium anisopliae* and *Beauveria bassiana*, other fungi such as *Isaria farinosa* (Holmsk) and *Isaria fumosorosea* (Wize) have been shown to be effective in combating cattle ticks, according to data from Angelo et al. (2012).

The results from in-vitro tests using the isolate IBCB 130 of *P. lilacinum* presented here have enabled field experiments with the aim of obtaining another candidate biological material for controlling *R. microplus* ticks.

AUTHORS' CONTRIBUTIONS

Conceptualization: Mendes, M.C.; Duarte, F.C.; Antonucci, Y.C.P.; Almeida, J.E.M. **Data curation:** Antonucci, Y.C.P.; Moura, T.A. **Formal analysis:** Mendes, M.C.; Duarte, F.C.; Antonucci, Y.C.P. **Investigation:** Antonucci, Y.C.P.; Moura, T.A. **Project administration:** Duarte, F.C.; Antonucci, Y.C.P.; Almeida, J.E.M. **Supervision:** Mendes, M.C.; Duarte, F.C. **Validation:** Mendes, M.C. Duarte, F.C.; Almeida, J.E.M. **Visualization:** Mendes, M.C.; Duarte, F.C.; Antonucci, Y.C.P. **Writing – original draft:** Mendes, M.C.; Duarte, F.C.; Antonucci, Y.C.P. **Writing – review & editing:** Mendes, M.C.; Duarte, F.C.; Antonucci, Y.C.P.; Almeida, J.E.M.; Moura, T.A.

AVAILABILITY OF DATA AND MATERIAL

All data generated or analyzed during this study are included in this published article.

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CONFLICTS OF INTEREST

Nothing to declare.

ETHICAL APPROVAL

Not applicable.

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