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# Efect of Extracts of Fungi Isolated from the Digestive Tract of Cattle on Larvae and Teleogynes of *Rhipicephalus microplus* Resistant to Cypermethrin

# Efeito de extratos de Fungos Isolados do Trato Digestório de Bovinos em Larvas e Teleóginas de *Rhipicephalus microplus* Resistentes a Cipermetrina

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**Abstract**
Controlling cattle ticks with conventional acaricides has favored resistance to acaricides, which has encouraged the search for effective and safe alternative methods of controlling this ectoparasite. Fungi have been used for biological control; however, little is known about the effectiveness of fungi from the bovine digestive tract in controlling cattle ticks. This work aimed to select fungi with active metabolites to control *Rhipicephalus microplus*. Those with extracts of *Trichoderma longibrachiatum* and *Aspergillus terreus* and the yeasts *Rhodotorula mucilaginosa* and *Pichia kudriavzevii* were evaluated. The extracts obtained from the fungi in Sabouraud Dextrose broth were analysed to Biocarrapaticidogram and larval package test (TPL) performed for two tick strains. For the Xangrilá tick strain, *T. longibrachiatum* (VN20) and *A. terreus* (VN15) extracts were evaluated, which showed low efficacy in controlling the reproductive activity of teleogynes and larvae mortality. For the ICAMG tick strain, extracts of *R. mucilaginosa* (isolates V10S and

V16S) and *P. kudriavzevii* (isolates V61 and V62) were evaluated. The filtrate from the cultivation of yeast V16S (R. mucilaginosa) at a low concentration (0.21 mg $mL^{-1}$) demonstrated 35% efficiency in reducing the reproductive activity of teleogynes. The results suggest the importance of future studies to evaluate the efficacy of higher concentrations and different formulations for the control of cattle ticks.

**Keywords**: *Rhipicephalus microplus.* Mycelial Fungi. Yeasts. Alternative Control. Acaricide Resistance.

**Resumo**
O controle do carrapato bovino com acaricidas convencionais tem favorecido a resistência a acaricidas, fomentando assim, a busca por métodos alternativos eficazes e seguros de controle desse ectoparasito. Fungos têm sido utilizados para o controle biológico, contudo pouco se conhece sobre a eficácia de fungos do trato digestório bovino no controle do carrapato bovino. O trabalho teve como objetivo selecionar fungos com metabólitos ativos para o controle de *Rhipicephalus microplus.* Os extratos de *Trichoderma longibrachiatum* e *Aspergillus terreus* e das leveduras *Rhodotorula mucilaginosa* e *Pichia kudriavzevii* foram obtidos a partir dos fungos em caldo Sabouraud Dextrose e então, foram avaliados o biocarrapaticidograma e teste de pacote de larvas (TPL) para duas cepas do carrapato. Para a cepa Xangrilá foram avaliados extratos *T. longibrachiatum* (VN20) e *A. terreus* (VN15) que apresentaram baixa eficácia sobre o controle da atividade reprodutiva das teleóginas e sobre a mortalidade de larvas. Para a cepa do carrapato ICAMG foram avaliados os extratos de *R. mucilaginosa* (isolados V10S e V16S) e *P. kudriavzevii* (isolados V61 e V62). O filtrado do cultivo da levedura V16S (*R. mucilaginosa*) em uma baixa concentração (0,21 mg $mL^{-1}$) demonstrou 35% de eficiência para redução da atividade reprodutiva das teleóginas. Os resultados sugerem a importância de futuros estudos para avaliar a eficácia em maiores concentrações e diferentes formulações para o controle do carrapato bovino.

**Palavras-chave:** *Rhipicephalus microplus*. Fungos Micelianos. Leveduras. Controle Alternativo. Resistência a Acaricida.

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## 1 Introducion

Dairy cattle farming is an activity of great social and economic importance in Brazil and worldwide. Minas Gerais is the state with the highest production, totaling more than 9.6 billion liters of milk per year (IBGE, 2022). Among the sanitary problems affecting cattle, the tick *Rhipicephalus microplus* is one of the main parasites that compromise herd productivity (Molento, 2020). Brazil's predominantly tropical climate, combined with extensive or semi-extensive farming systems, favors the tick's life cycle, which depends on periods of heat and humidity, as well as the presence of pasture and host animals (Vaz Júnior *et al*., 2012).

In addition to the damage caused by hematophagy, ticks act as vectors of *Anaplasma marginale, Babesia bigemina,* and *Babesia bovis,* the causative agents of bovine parasitic sadness (Molento, 2020). They also cause skin lesions in cattle and lead to high costs for medications (Grisi *et al*., 2014). Another risk associated with this ectoparasite is the irrational and constant use of

tickicides by farmers, which induces the selection of resistant populations to the main acaricides available (Abbas *et al*., 2014; Dolenga *et al*., 2017; Klafke *et al*., 2017).

An analysis of acaricide efficacy over a 20-year period in Brazil indicated that some groups of acaricides showed high tick resistance, achieving less than 20% effectiveness (Embrapa, 2020). Moreover, residues from these chemical acaricides can contaminate animal-derived products such as milk and meat, the environment, and workers handling livestock (Laing *et al*., 2018; Welsh *et al*., 2019).

Studies on biological control using fungi have shown promising results in tick management (Samish *et al*., 2014). Jones (2017) and Muniz *et al*. (2020) reported promising results with the use of *Metarhizium* sp. conidia on larvae and engorged females of *Rhipicephalus* spp. Other studies have demonstrated the efficacy of *Beauveria bassiana* in the mortality of *R. microplus* engorged females (Rivera *et al*., 2018; Sun *et al*., 2013).

Other fungal genera have also shown potential in parasite control and are easily found in various environments, such as *Trichoderma* spp., *Aspergillus* spp., *Rhodotorula* spp., and *Pichia* spp. (Chan *et al*., 2012; Liu *et al*., 2013; Miranda-Miranda *et al*., 2012; Singh *et al*., 2017). Fungi of these genera present in the digestive tract of cattle at concentrations exceeding $10^6$ colony-forming units (CFU) per mL have been isolated and identified (Abrão *et al*., 2014; Duarte *et al*., 2024).

The potential of extracts produced from these fungi and used for cattle tick control is still unknown. Therefore, this study aimed to investigate the acaricidal efficacy of extracts from the fungi *Trichoderma longibrachiatum* and *Aspergillus terreus,* as well as the yeasts *Rhodotorula mucilaginosa* and *Pichia kudriavzevii,* obtained from the cattle's digestive tract, on larvae and engorged females of *R. microplus.*

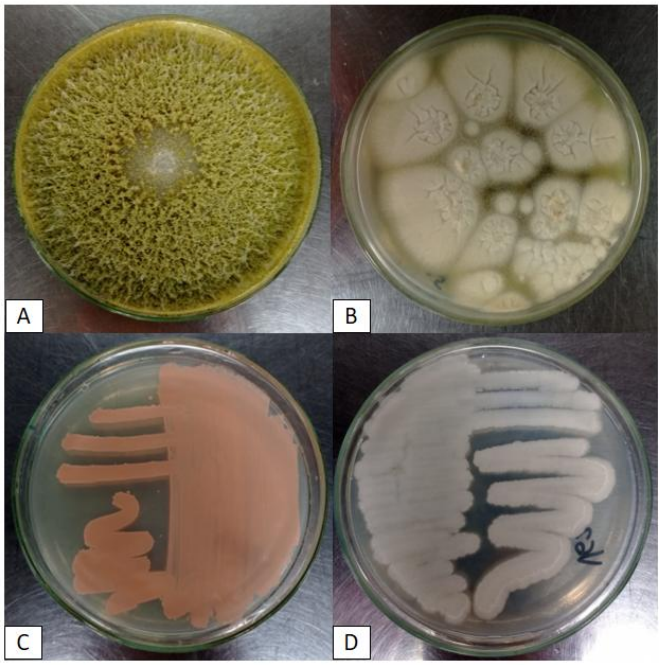
## 2 Material and Methods

### 2.1 Fungal isolates used

The VN20 isolate of *Trichoderma longibrachiatum* [KF781535] (Figure 1a) and VN15 isolate of *Aspergillus terreus* [KF781532] (Figure 1b) were obtained from the digestive tract of Nelore steers raised under a grazing system with *Urochloa decumbens* and supplemented with a mineral

mixture (Abrão et al., 2014). The yeast-like isolates V10S and V16S (*Rhodotorula mucilaginosa*) [KU316790.1] (Figure 1c) and V61 and V62 (*Pichia kudriavzevii*) [KU316741.1] (Figure 1d) were extracted from the ruminal fluid of Nelore cows raised on *Urochloa brizantha* (Marandu grass) pasture and identified as described by Duarte *et al*. (2024).

**Figure 1 -** Fungal isolates cultured on Sabouraud agar. a – Trichoderma longibrachiatum. b – Aspergillus terreus. c – Rhodotorula mucilaginosa. d – Pichia kudriavzevii. (Personal archive)




**Source**: research data.

The molecular identification of the mycelial fungi was performed by sequencing ribosomal DNA (Abrão et al., 2014). The ITS region of rDNA was amplified by polymerase chain reaction (PCR) using the primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (White *et al*., 1990). The sequencing reactions were carried out using DYEnamic™ (Amersham Biosciences, USA) in conjunction with the MegaBACE™ 1000 automated sequencing system. The obtained sequences were trimmed using BioEdit software Version 7.2.5 and Asparargim (http://asparagin.cenargen.embrapa.br/phph/) to remove low-quality regions (Phred Score <20), and the assembly of nucleotide sequence contigs was edited and

compared with sequences in the GenBank database (http://www.ncbi.nlm.nih.gov/) using the Blast N program (Altschul *et al.,* 1997).

The yeasts were identified through sequence analysis of the D1 and D2 domains of the 26S ribosomal RNA gene, amplified by PCR (Duarte et al., 2024). Total DNA extraction was performed (Hoffman; Winston, 1987), and the primers NL1 (5'-GCA TAT CAA AAG GAA GAG TAA GCC-3') and NL4 (5'-GGT AAG CTT CGC TGT CCG G-3') were used for the amplification of the rDNA region, following the methodology described by Burgaud *et al*. (2013), with modifications. The nucleotide sequences obtained using the 3730xl DNA Analyzer (Applied Biosystems) were edited and compared with sequences deposited in GenBank using the Blast N program (Altschul *et al*., 1997).

The mycelial fungi were stored in sterile vials containing sterile cellulose paper discs with spores, while the yeasts were stored in Sabouraud Dextrose broth (KASVI®, Teramo, Italy) in an ultra-freezer at -80 ºC.

**2.2 Obtaining fungal extracts**

Discs containing spores of the mycelial fungi were inoculated onto Petri dishes with Sabouraud Dextrose Agar (KASVI®, Teramo, Italy) and incubated in a BOD chamber at 37 °C for seven days. The spores from pure colonies were scraped and suspended in 0.85% saline solution with the addition of 0.01% Tween 80 (Freitas, 2018).

For cultivation, 400 mL of Sabouraud Dextrose broth (KASVI®, Teramo, Italy) were prepared, standardized at $10^8$ CFU $mL^{-1}$, and incubated in a benchtop shaker incubator (NT712, Novatecnica, São Paulo, Brazil) at 39 °C and 120 rpm for seven days. After this period, the medium containing the grown fungi was blended in an industrial blender, homogenized, and filtered through gauze and cotton (Nery et al., 2010), then stored in an ultra-freezer at -80 ºC. To measure the dry matter (DM) content, 30 mL samples of the filtrates were placed on Petri dishes and dried in an oven at 105 °C until weight stabilization. The filtrate of isolate VN20 had a DM content of 2.22%, while isolate VN15 had 1.54% DM.

The yeasts were cultured on Sabouraud Dextrose Agar (KASVI®, Teramo, Italy) supplemented with chloramphenicol (150 mg $L^{-1}$) for two days at 37 °C. The colonies were suspended in 0.85% saline solution with 0.01% Tween 80 and standardized at $10^9$ CFU $mL^{-1}$. Subsequently, 10 mL were inoculated into 100 mL of Sabouraud Dextrose broth (KASVI®, Teramo, Italy) and incubated in a benchtop shaker incubator (NT712, Novatecnica, São Paulo, Brazil) at 39 ºC and 120 rpm for three days.

To obtain the extracts, the media were centrifuged at 270g for 10 minutes (8BTF, ITR, Rio Grande do Sul, Brazil), and the supernatant was collected using a glass pipette and stored in an ultra-freezer at -80 ºC. The DM contents were 2.26% for V10S, 2.11% for V16S, 1.01% for V61, and 1.05% for V62.

### 2.3 Evaluated *Rhipicephalus microplus* populations

The Xangrilá strain was isolated from dairy cattle raised in paddocks with *Brachiaria purpuracens* (capim-bengo) grass, irrigated using a flood system. The ICAMG strain was obtained from dairy cattle raised in a semi-confined system with grazing on *Urochloa brizantha*. For both strains, samples were collected from crossbred animals (¾ Holstein, ¼ Gir) on farms located in Montes Claros, Minas Gerais, that had not received any tickicide treatment for at least 60 days prior to collection.

This study was approved by the Ethics Committee on Animal Use of the Federal University of Minas Gerais (CEUA - UFMG) and registered under protocol number 265/2017.

### 2.4 Immersion of engorged females in fungal extracts

The bioacaricidogram test was conducted following the methodology proposed by Drummond *et al*. (1973). For the Xangrilá strain, the *in vitro* efficacy of filtrates from the mycelial fungal isolates VN20 and VN15 was evaluated and compared with the main acaricides used on the property, as well as a control group containing only distilled water. For the groups treated with fungal filtrates, 1 mL of the filtrate was used per engorged female.

The commercial acaricides evaluated for the Xangrilá strain included cypermethrin at 0.15 mg $mL^{-1}$ (Barrage, Zoetis, São Paulo, Brazil), supona at 0.05 mg $mL^{-1}$ (Carrapaticida e Sarnicida UCB, Uzinas Chimicas Brazileiras, São Paulo, Brazil), deltamethrin at 25 mg $mL^{-1}$ (Butox P CE 25, MSD, São Paulo, Brazil), a combination of cypermethrin at 150 mg $mL^{-1}$, chlorpyrifos at 250 mg $mL^{-1}$, and citronellal at 10 mg $mL^{-1}$ (Colosso Pulverização, Ourofino Saúde Animal, São Paulo, Brazil), and amitraz at 0.25 mg $mL^{-1}$ (Triatox, MSD Saúde Animal, São Paulo, Brazil), diluted according to the manufacturers' recommendations.

For the ICAMG strain, the supernatant from the culture of isolates V10S, V16S, V61, and V62 was used, with 1 mL per engorged female in each replicate. The commercial acaricides evaluated were supona [(2-chloro-1-(2,4-dichlorophenyl) vinyl diethyl phosphate] at 0.05 mg $mL^{-1}$ (Carrapaticida e Sarnicida UCB, Uzinas Chimicas Brazileiras, São Paulo, Brazil), a combination of cypermethrin at 150 mg $mL^{-1}$, chlorpyrifos at 250 mg $mL^{-1}$, and citronellal at 10 mg $mL^{-1}$ (Colosso Pulverização, Ourofino Saúde Animal, São Paulo, Brazil), and amitraz at 0.25

mg $mL^{-1}$ (Triatox, MSD Saúde Animal, São Paulo, Brazil), diluted according to the manufacturers' recommendations. A control group containing only distilled water was also included.

The engorged females were washed in running water, dried with paper towels, and distributed into homogeneous groups. Each group had four replicates, with each replicate containing five engorged females, which were placed in Petri dishes and weighed on an analytical balance.

The groups of engorged females were submerged for 5 minutes in 5 mL of their respective treatment solutions. After this period, they were dried with paper towels and incubated in a BOD chamber at a temperature of 28 ± 1°C and relative humidity close to 80% for 15 days for oviposition. On the 15th day, the weight of the egg masses was measured using an analytical balance. The egg masses were placed in disposable syringes sealed with hydrophilic cotton and incubated under the same temperature and humidity conditions for 21 days.

To assess hatchability (EC), the methodology proposed by Figueiredo *et al*. (2019) was applied with modifications. A solution of water and neutral detergent in a 1:1 ratio was added to the syringes containing larvae and eggs. After vigorous shaking, 200 µL aliquots of the suspension were analyzed on slides under a light microscope at 10X magnification to count larvae and eggs.

The following parameters were evaluated (Bennett, 1974; Drummond *et al*., 1973): oviposition capacity (CP), EC, reproductive efficiency (ER), and product efficacy (EP), calculated using the following formulas:

- CP = (egg mass weight / engorged female weight) × 100
- EC = number of larvae / (number of eggs + number of larvae)
- ER = (egg mass weight × EC × 20,000) / engorged female weight
- EP = ((ER of the control group - ER of the treated group) / ER of the control group) × 100

**2.5 Larval packet test**

After 21 days from the onset of egg hatching in both strains, the Larval Packet Test (LPT) was conducted (Stone; Haydock, 1962). Packets were made using filter paper (Whatman No. 1) with dimensions of 6 × 6 cm, sealed on the sides while keeping one side open for larval insertion. Each group had four replicates, with 100 larvae per replicate.

The larvae of each strain were placed inside the packets using a fine brush, the packets were sealed with clips, and 400 µL of each test solution was applied. The extracts from the same fungi

described previously were evaluated, along with a control group treated with the chemical acaricide supona at 0.05 mg $mL^{-1}$ (Carrapaticida e Sarnicida UCB, Uzinas Chimicas Brazileiras, São Paulo, Brazil) and a control group with distilled water. The treatment group packets were placed in Petri dishes and incubated under the same conditions described for engorged females for 24 hours. After this period, the number of live and dead larvae was counted.

### 2.6 Statistical analysis

The evaluations were conducted using a completely randomized design for the evaluated tick strain. The data were subjected to analysis of variance (ANOVA) among the groups treated with commercial acaricides, fungal extracts, and controls, and the means were compared using the Scott-Knott test at a 5% probability level, utilizing the SAEG statistical package (*Sistema para Análises Estatísticas*, Version 9.1: Fundação Arthur Bernardes - UFV - Viçosa, 2007).

## 3 Result and Discussion

### 3.1 Effect of Fungal Filtrates on the Reproductive Activity of Teleogynes Xangrilá Strain

The filtrates from the mycelial fungal cultures VN15 and VN20, evaluated for the Xangrilá strain, did not reduce oviposition capacity (OC) or hatchability (H). The administration of amitraz and supona resulted in lower OC and H, as well as higher acaricidal efficacy compared to the other evaluated products (Table 1).

**Table 1 -** Effect of the Filtrates from Trichoderma longibrachiatum (Isolate VN20) and Aspergillus terreus (Isolate VN15) Cultures on the Reproductive Activity of Engorged Females of *Rhipicephalus microplus* (Xangrilá Strain)

| Treatments | Oviposition Capacity (%)* | Hatchability (%) | Product Efficiency (%)** |
|---|---|---|---|
| Isolate VN20 | 50.33a ±2.83 | 97.62a ±2.12 | 6.73d ±5.62 |
| Isolate VN15 | 52.15a ±5.78 | 97.88a ±1.15 | 5.63d ±8.79 |
| Cypermethrin | 40.67b ±9.33 | 97.77a ±0.94 | 25.15c ±9.86 |
| Deltamethrin | 44.02b ±12.23 | 87.01a ±3.58 | 27.49c ±19.84 |
| Combination[a] | 22.51c ±4.63 | 58.21b ±8.60 | 75.12b ±7.83 |
| Supona | 5.00d ±4.32 | 17.48c ±23.45 | 97.28a ±5.50 |
| Amitraz | 9.16d ±5.82 | 33.67c ±36.59 | 92.22a ±8.54 |
| Control | 52.98a ±2.87 | 98.81a ±1.09 | - |

[a]Combination (product containing cypermethrin, chlorpyrifos, and citronellal).* Oviposition Capacity (OC) = (egg mass weight / initial female weight) × 100. **Product Efficiency (PE) = [(Reproductive Efficiency of Control - Reproductive Efficiency of Product) / Reproductive Efficiency of Control] × 100. Means (± standard deviation) followed by the same letter in the columns are statistically similar according to the Scott-Knott test at a 5% significance level.
**Source:** research data.

### 3.2 Effect of Fungal supernatants on the reproductive activity of teleogynes – ICAMG Strain

The supernatants from the yeasts evaluated in teleogynes of the ICAMG strain were not effective in reducing OC and H. However, the supernatant from yeast V16S reduced the reproductive efficiency (RE) of the tick compared to the control group ($p<0.01$) and showed a PE of 35% (Table 2). The use of Supona resulted in the greatest reduction in OC and H among the evaluated acaricides.

**Table 2 -** Effect of Supernatants from *Rhodotorula mucilaginosa* (Isolates V10S and V16S) and *Pichia kudriavzevii* (Isolates V61 and V62) Cultures on the Reproductive Activity of Engorged Females of *Rhipicephalus microplus* (ICAMG Strain)

| Treatments | Oviposition Capacity (%)* | Hatchability (%) | Product Efficiency (%)** |
|---|---|---|---|
| Isolate VN20 | 42.89a ±4.95 | 92.99a ±2.89 | 1.20d ±2.07 |
| Isolate VN15 | 33.03a ±11.34 | 63.76b ±15.94 | 35.00c ±30.23 |
| Cypermethrin | 38.86a ±1.72 | 88.63a ±5.70 | 5.79d ±6.14 |
| Deltamethrin | 44.07a ±9.06 | 89.06a ±4.07 | 3.66d ±5.18 |
| Combination[a] | 18.63b ±4.06 | 78.46a ±9.85 | 59.37b ±3.22 |
| Supona | 18.04b ±3.75 | 83.52a ±4.54 | 55.99b ±11.29 |
| Amitraz | 7.62b ±5.39 | 38.20c ±27.01 | 83.37a ±11.79 |
| Control | 40.05a ±4.77 | 86.47a ±14.11 | - |

[a]Combination of cypermethrin, chlorpyrifos, and citronellal.
**Oviposition Capacity (OC) = (egg mass weight / initial female weight) × 100.*
***Product Efficiency (PE) = [(Reproductive Efficiency of Control - Reproductive Efficiency of Product) / Reproductive Efficiency of Control] × 100.Means (± standard deviation) followed by the same letter in the same column are statistically similar according to the Scott-Knott test at a 5% significance level.
**Source:** research data.

### 3.3 Larval Mortality – Xangrilá Strain

The filtrates from VN20 and VN15 caused low mortality in the larvae of the Xangrilá strain. The chemical treatment with Supona, which showed the highest efficacy among acaricides in the bioacaricide assay for the tested strain, resulted in 100% larval mortality (Table 3).

**Table 3 –** Larval Mortality (%) of *Rhipicephalus microplus* (Xangrilá Strain) Treated with Filtrates from *Trichoderma longibrachiatum* (Isolate VN20) and *Aspergillus terreus* (Isolate VN15)

| Treatments | Mortality (%) |
|---|---|
| Isolate VN20 | 3.39b ±3.45 |
| Isolate VN15 | 2.78b ±2.02 |
| Supona | 100.00a ±0 |
| Control | 11.74 b ±8.18 |

Means (± standard deviation) followed by the same letter in the column are statistically similar according to the Scott-Knott test at a 5% significance level.
**Source:** Research data.

### 3.4 Larval Mortality – ICAMG Strain

In all treatments with yeast isolates on the tick larvae of the ICAMG strain, low larval mortality was observed. Similarly to the Xangrilá strain, Supona caused 100% mortality of the tested larvae.

**Table 4 –** Larval Mortality (%) of *Rhipicephalus microplus* (ICAMG Strain) Treated with Supernatant from *Rhodotorula mucilaginosa* (Isolates V10S and V16S) and *Pichia kudriavzevii* (Isolates V61 and V62)

| Treatments | Mortality(%) |
|---|---|
| V10S | 13.42b ± 8.01 |
| V16S | 16.29b ±12.24 |
| V61 | 4.78b ±6.50 |
| V62 | 16.52b ±17.00 |
| Supona | 100.00a ±0.0 |
| Control | 11.74b ±8.18 |

Means (± standard deviation) followed by the same letter in the column are statistically similar according to the Scott-Knott test at a 5% significance level.
**Source:** research data.

The bioacaricide assay analysis for cypermethrin, deltamethrin, and the combination of cypermethrin, chlorpyrifos, and citronellal indicated resistance in the Xangrilá strain, with a PE below 95%, as specified by Brazilian legislation (Brasil, 2012). Supona and amitraz showed similar efficacy and were the most effective acaricides for the evaluated strain, making them the recommended products for the strategic control of this strain.

When evaluating the effects of fungal extracts on tick larvae and teleogynes, no differences were observed compared to the control group. The low acaricidal efficacy of these bioproducts in the present study may be related to the low concentration of metabolites, as they exhibited low dry matter content ($\leq$0.22 mg $mL^{-1}$). Conversely, Zahran *et al.* (2017) achieved 93% mortality of tropical bedbugs (*Cimex hemipterus*) using spore suspensions of *Aspergillus tubingensis* and *Trichoderma harzianum*, demonstrating that spore suspensions may yield better results compared to filtrates.

Studies on *Trichoderma* spp. have demonstrated their ability to produce enzymes and act against insect defense mechanisms (Mach; Zeilinger, 2003; Mukherjee *et al*., 2008). *Aspergillus* spp. can also cause damage to insects through the production of metabolites that adhere to the cuticle (Shahid *et al*., 2012). However, in the present study, fungal extracts from these genera did not exhibit acaricidal effects.

The *Trichoderma* genus comprises fungi with entomopathogenic potential, capable of competing for substrates, exhibiting antibiosis and parasitism, and being easily found in soil and the

animals' digestive tract, indicating their wide bioavailability (Bernardo *et al*., 2019; Brito *et al*., 2010; Pavani, 2017). Maia Filho *et al*. (2017) reported the presence of *Trichoderma virens* hyphae in *Toxocara canis* eggs after exposure to fungal mycelia, with a 57.4% reduction in egg hatchability in the exposed group compared to the control group. Vieira *et al*. (2016), using *Trichoderma longibrachiatum*, observed that the fungal filtrate achieved 92.8% efficacy in larval mortality of *Haemonchus contortus*. These fungal actions were attributed to the production of chitinases, which contribute to the degradation of the external structures of these helminth eggs.

Aspergillus spp. are also found in various soil types and the animals' digestive tracts. Many species of this genus can produce extracellular enzymes, acids, and secondary metabolites, allowing for diverse environmental and industrial applications (Martins Junior *et al.,* 2023; Ward *et al.,* 2005*)*. In biological control, Djouhri *et al*. (2022) demonstrated the efficacy of *A. terreus* at a concentration of $10^8$ spores mg $mL^{-1}$ in the mortality of Blattella germanica cockroaches, achieving a mortality rate of 92.96% in adults through direct contact with the spore solution.

For the ICAMG strain, all the chemical acaricides tested on engorged females indicated resistance, according to the parameters established by the Brazilian legislation (Brasil, 2012). Although Supona demonstrated a high capacity for larval mortality in the tested strain, its efficacy remained below 84%. Thus, the ICAMG strain can be considered multidrug-resistant, highlighting the need for alternative acaricides or bioproducts for effective control.

The results obtained from the V16S yeast supernatant showed potential in reducing oviposition capacity (CP) and egg hatchability (EC), resembling the effects of chemical treatments with amitraz and the combination of cypermethrin, chlorpyrifos, and citronellal. This effect could be associated with the production of lipases and proteases by the yeast (Duarte *et al*., 2013). Seifi *et al*. (2016) reported lipase production in various *Rhodotorula* species, including *R. mucilaginosa*. These enzymes may have acted on the external and internal structures of the engorged females, either by degrading the cuticle or penetrating the ticks' natural openings, following the mechanisms of action of contact acaricides as described by Furlong *et al*. (2007).

It was also possible to observe intraspecific variation, as another isolate of the same species, *R. mucilaginosa*, showed inferior results in the *in vitro* control of the evaluated tick strain. These findings highlight the importance of strain selection for biological control to achieve greater effectiveness due to phenotypic and genetic heterogeneity, as observed by Libkind *et al*. (2008) in *R. mucilaginosa* strains and by González-Arenzana *et al*. (2017) in various other yeast species.

The supernatants of isolates V10S, V61, and V62 showed low potential in reducing CP and EC and, consequently, demonstrated low EP. The supernatants of isolates V61 and V62 had lower MS levels when compared to the *R. mucilaginosa* isolates, which may be related to the

concentration of metabolites present in the supernatant. Lata *et al*. (2022) studied an isolate of *P. kudriavzevii* and observed its potential to produce various enzymes, such as β-galactosidase, protease, amylase, phytase, and lipase. However, in the present study, the effects of these enzymes were not significantly observed in the *in vitro* control of the evaluated tick strain.

## 4 Conclusion

The supernatant of the *R. mucilaginosa* V16S isolate has potential in reducing the reproductive activity of the *R. microplus* ICAMG strain, which is multiresistant to conventional acaricides.

The extracts and filtrates of the other evaluated fungi did not show efficacy in the *in vitro* control of the assessed tick strains.

Future studies should evaluate the composition and different formulations or concentrations of the metabolites produced by the V16S yeast isolate in the control of ticks and other mites.

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