

Activity *in vitro* of fungal conidia of *Duddingtonia flagrans* and *Monacrosporium thaumasium* on *Haemonchus contortus* infective larvae

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Abstract

The objective of this work was to evaluate the predatory activity of the fungi *Duddingtonia flagrans* (AC001) and *Monacrosporium thaumasium* (NF34a) on *Haemonchus contortus* infective larvae (L3) in two experimental assays (A and B). In assay A, two treatments and one control were formed and kept for 7 days in Petri dishes with 2% water-agar. Each treatment consisted of 1000 *H. contortus* L3 and 1000 conidia of only one fungal isolate, and the control group consisted of 1000 L3, without fungus, with 10 repetitions per group. In assay B, 1000 conidia of one of the fungal isolates, AC001 or NF34a, were added to coprocultures made from 20 g of faeces collected from sheep naturally infected with *H. contortus*. At the end of the experiment, the Baermann method was used to count the non-predated larvae of all Petri dishes from treatment and control groups. In assay A, no difference was observed ($P > 0.05$) between the groups treated with AC001 and NF34a fungi. A difference was observed ($P < 0.05$) between the treated and control groups. The L3 reduction percentages at the end of the experiment were 87.75 and 85.57%, respectively, for the fungal isolates compared to the control group. In assay B, the reduction percentages for conidia of these isolates were 85.82 and 87.32%, respectively. The results obtained show that *D. flagrans* (AC001) and *M. thaumasium* (NF34a) were effective in the *in vitro* control of sheep *H. contortus* L3 and could be used in the biological control of this nematode.

Introduction

Significant economic losses in sheep production are associated with parasitism by gastrointestinal helminths, mainly *Haemonchus contortus*. The annual world economic losses due to infections caused by gastrointestinal nematodes are estimated in millions of dollars (Amarante *et al.*, 2009; Silva *et al.*, 2009). In addition, the frequent use of anthelmintics for the prophylaxis of gastrointestinal nematode (GIN) infections has led to the dissemination of populations of resistant parasites (Getachew *et al.*, 2007). Research worldwide looks for alternative measures for control of domestic animal helminthiasis, aiming to

reduce the usage of chemotherapeutics (Silva *et al.*, 2009). The application of biological control using nematophagous fungi has become a viable alternative and has presented itself as promising. Some work done with the species *Duddingtonia flagrans* and *Monacrosporium thaumasium* demonstrate their effectiveness in the control of nematodes in the laboratory (Waller *et al.*, 1994; Larsen, 1999; Terril *et al.*, 2004). The objective of this work was to evaluate *in vitro* activity of fungal conidia of *D. flagrans* and *M. thaumasium* on *H. contortus* infective larvae.

Materials and methods

Fungal cultures and experimental assays

The isolates of the predator fungal species *D. flagrans* (AC001) and *M. thaumasium* (NF34a) were kept in test

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tubes containing 2% corn–meal–agar (CMA), in the dark, at 4°C for 10 days. The isolates were previously stored at the Laboratory of Parasitology in the Department of Veterinary Medicine, Federal University of Viçosa, Minas Gerais, Brazil. Petri dishes containing 2% water-agar (2% WA) were inoculated with one of the isolates and incubated at 26°C for 10 days. After that, the conidia were collected according to Araújo *et al.* (1993).

The present work consisted of two experimental assays (A and B). In assay A, the predatory activity of the predator fungi *D. flagrans* and *M. thaumasium* on *H. contortus* L3 was evaluated in an *in vitro* assay on Petri dishes containing 2% WA. In assay B, the predatory capacity of the fungal conidia of the isolates *D. flagrans* and *M. thaumasium* on sheep faeces naturally infected by *H. contortus* was evaluated.

Collection of L3 of *Haemonchus contortus*

The *H. contortus* L3 were obtained by the Baermann method after coprocultures were carried out for 10 days, according to Gordon & Whitlock (1939), using naturally infected sheep faeces that were positive for the superfamily Strongyloidea. Using light microscopy ($\times 10$ objective lens) the samples with 100% *H. contortus* were used in the experiment, having been washed with distilled water and centrifuged five times to remove the supernatant. The coprocultures were carried out, for each sheep, according to the methodology described by Roberts & O'Sullivan (1950). The identification of the infective larvae in the coprocultures was performed according to Keith (1953).

Collection of conidia

Culture discs (4 mm in diameter) were removed from the fungal isolates kept in test tubes containing 2% CMA and transferred to 9.0-cm Petri dishes containing 20 ml of 2% potato dextrose agar and kept at 25°C in the dark for 10 days. After growth, new culture discs (4 mm in diameter) were transferred to 9.0-cm diameter Petri dishes containing 20 ml of 2% water-agar (2% WA) and 1 ml of distilled water containing 1000 larvae of *Panagrellus* sp. was added daily for 21 days to induce conidia formation. When fungal development was complete, 5 ml of distilled water were added to each Petri dish, and the conidial and mycelial fragments were removed as described by Araújo *et al.* (1993).

Assay A

Two treatments and one control were made and kept at 26°C for 7 days. Each treatment consisted of 1000 conidia of a fungal isolate and 1000 *H. contortus* L3, with 1000 L3 without fungus as the control, and ten repetitions per group. Ten random fields of each plate were counted daily, checking the number of predated larvae until the end of the experiment (seventh day). After the last reading of the plates, the Baermann method was used to count the non-predated larvae of all treated groups and control plates.

Assay B

In coprocultures made from 20 g of faeces from sheep naturally infected by *H. contortus*, 1000 conidia of the fungal isolates *D. flagrans* (AC001) and *M. thaumasium* (NF34a) were homogenized and plated. In the control group, only coprocultures made from 20 g of faeces from sheep naturally infected by *H. contortus* were used. There were six replicates per group. At the end of 7 days, the *H. contortus* larvae were recovered from the treated and control groups using the Baermann method, according to the technique described by Araújo *et al.* (1993).

Data analysis

Means of recovered *H. contortus* L3 were calculated. Data were examined by analysis of variance at significance levels of 1 and 5% probability (Ayres *et al.*, 2003). Predation efficiency of L3 relative to the control group was evaluated by the Tukey's test at 5% probability. The reduction percentage of L3 means was calculated according to the following equation: Reduction % = ((Mean of L3 recovered from control – Mean of L3 recovered from treatment)/Mean of L3 recovered from control) \times 100.

Results and discussion

The mean of non-predated *H. contortus* L3 per 4 mm diameter field during the experiment is shown in table 1. The *D. flagrans* (AC001) and *M. thaumasium* (NF34a) fungal isolates predated the larvae throughout the experimental assay. The predation was observed on the first reading of the two treated groups, 24 h after the interaction between the larvae and the fungal isolates. However, the presence of fungus and formed traps predated the L3 were not observed in the control group Petri dishes.

At the end of the experimental assay, no difference was found ($P > 0.05$) between the groups treated with the fungi *D. flagrans* (AC001) and *M. thaumasium* (NF34a); nonetheless, differences were observed between the treated and the control groups (fig. 1). The reduction percentages of the L3, obtained using the Baermann

Table 1. Daily means and (\pm) standard error of non-predated infective larvae (L3) of *Haemonchus contortus* per 4 mm diameter field in 2% water-agar during 7 days of treatment with the fungal isolates *Duddingtonia flagrans* (AC001), *Monacrosporium thaumasium* (NF34a) and control without fungus.

Time (days)	Treatment (mean of L3 non-predated)		
	AC001	NF34a	Control
1	1.33 \pm 1.93 ^A	1.83 \pm 2.05 ^A	5.85 \pm 5.83 ^B
2	0.77 \pm 1.20 ^A	1.8 \pm 1.59 ^A	5.07 \pm 4.72 ^B
3	0.65 \pm 0.95 ^A	4.85 \pm 2.78 ^B	15.1 \pm 6.92 ^C
4	1.23 \pm 1.83 ^A	6.87 \pm 3.09 ^B	22.53 \pm 7.69 ^C
5	0.83 \pm 1.55 ^A	0.78 \pm 0.76 ^A	12.1 \pm 5.96 ^B
6	0.58 \pm 1.06 ^A	0.43 \pm 0.56 ^A	9.92 \pm 4.83 ^B
7	0.25 \pm 0.47 ^A	0.02 \pm 0.13 ^A	14.88 \pm 6.30 ^B

Means followed by the same letter in the same column are not significantly different ($P > 0.05$) by Tukey's test.

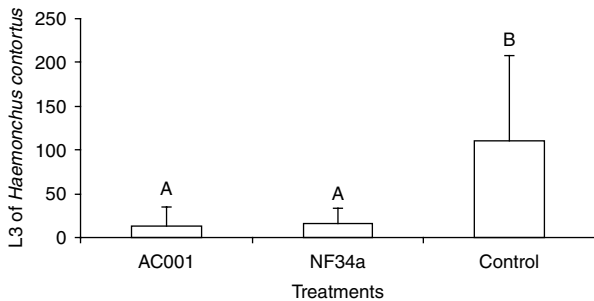


Fig. 1. Mean number of non-predated *Haemonchus contortus* infective larvae (L3) recovered in 2% water-agar by the Baermann method on the seventh day after interaction with the fungal isolates *Duddingtonia flagrans* (AC001), *Monacrosporium thaumasium* (NF34a) and control (without fungus). Lines on bars represent standard deviations. Means accompanied by at least one common capital letter (A) are not significantly different by Tukey's test at a 5% probability level.

method at the end of the experiment, with the fungi *D. flagrans* (AC001) and *M. thaumasium* (NF34a), were 87.75 and 85.57%, respectively, compared to the control group.

At the end of 7 days, the conidia of *D. flagrans* (AC001) and *M. thaumasium* (NF34a) isolates reduced the number of recovered L3 from Assay B. However, no difference ($P < 0.05$) was found between the treatments. Moreover, a difference ($P < 0.05$) was observed between the treatments and the control group (fig. 2). The reduction percentages due to the *D. flagrans* (AC001) and *M. thaumasium* (NF34a) isolates were 85.82 and 87.32%, respectively.

In the presence of nematodes, the fungi are able to produce traps in a short period of time. This predation was already seen in the first reading of the Petri dishes of the treated groups 24 h after the interaction between the larvae and the fungal isolates. However, the presence of fungus and of formed traps predating the L3 present in the control group Petri dishes was not observed.

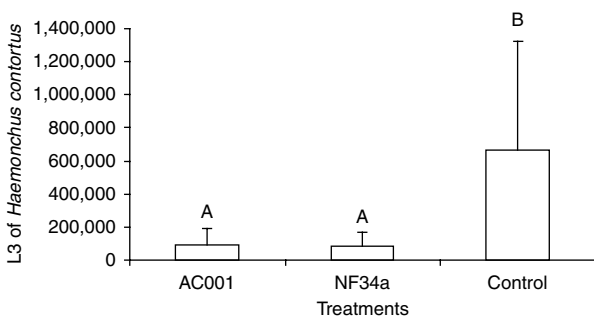


Fig. 2. Mean number of non-predated *Haemonchus contortus* infective larvae (L3) recovered in coprocultures by the Baermann method on the seventh day after interaction with the fungal isolates *Duddingtonia flagrans* (AC001), *Monacrosporium thaumasium* (NF34a) and control (without fungus). Lines on bars represent standard deviation. Means accompanied by at least one common capital letter (A) are not significantly different by Tukey's test at a 5% probability level.

During the present work, the temperature was kept constant at 26°C, and it was observed that there was an effective reduction of the number of *H. contortus* L3 due to the presence of *D. flagrans* and *M. thaumasium* fungal isolates throughout and after 7 days of interaction (table 1 and fig. 1). According to Gronvold *et al.* (1996), after the capture of the larvae by the trapping structures of the predatory fungi, a process of hyphal penetration into the cuticle occurs, followed by the digestion of the nematodes' interior. The most commonly observed differentiated structures of these fungi along the mycelium are non-adhesive constricting rings, non-constricting rings, buttons, and three-dimensional adhesive networks. These results are in accordance with the present work, since the production of traps on *H. contortus* L3 was observed in the experimental assay.

Furthermore, *M. thaumasium* (NF34a) destroyed the *H. contortus* L3, with a reduction of 85.57% of the larvae at the end of the experimental assay, demonstrating its effectiveness. Fungi of the genus *Monacrosporium* (*M. sinense* and *M. thaumasium*), have been evaluated by several authors, demonstrating effectiveness in the control of gastrointestinal nematodiosis of different animal species (Araújo *et al.*, 1992; Braga *et al.*, 2009).

In assay B, a reduction of the number of *H. contortus* L3 recovered from the faeces was observed, caused by conidia of the *D. flagrans* (AC001) and *M. thaumasium* (NF34a) isolates. These results are in accordance with Silva *et al.* (2009) who also observed that these isolates could be used in the control of sheep haemonchosis in natural conditions. In the same work, *H. contortus* was the most prevalent gastrointestinal parasitic nematode, demonstrating its importance in sheep production.

The results obtained demonstrated that fungal conidia of *D. flagrans* (AC001) and *M. thaumasium* (NF34a) were effective in the *in vitro* control of sheep *H. contortus* infective larvae (L3) and could be used in the biological control of this nematode.

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