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In vitro ovicidal activity of the nematophagous fungi *Duddingtonia flagrans*, *Monacrosporium thaumasium* and *Pochonia chlamydosporia* on *Trichuris vulpis* eggs

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ABSTRACT

The *in vitro* effect of four isolates of the nematophagous fungi *Duddingtonia flagrans* (AC001), *Monacrosporium thaumasium* (NF34a) and *Pochonia chlamydosporia* (VC1 and VC4) on the eggs of *Trichuris vulpis* was evaluated. One thousand eggs of *T. vulpis* were plated on Petri dishes with 2% water–agar with the fungal isolates grown and without fungus as control. After 7, 14 and 21 days 100 eggs were removed from each plate and classified according to the following parameters: type 1, lytic effect without morphological damage to eggshell; type 2, lytic effect with morphological alteration of embryo and eggshell; and type 3, lytic effect with morphological alteration of embryo and eggshell, besides hyphal penetration and internal egg colonization. *P. chlamydosporia* demonstrated ovicidal activity ($p < 0.05$) on the eggs of *T. vulpis* in the studied intervals presenting type 3 effect of 29.5% (VC1) and 36.5% (VC4), 59.5% (VC1) and 2.5% (VC4), 94.8% (VC1) and 2.95% (VC4) at 7, 14 and 21 days, respectively. The other fungi showed no type 3 effect. *P. chlamydosporia* should be a potential biological control agent of *T. vulpis* eggs.

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1. Introduction

The gastrointestinal parasite nematode *Trichuris vulpis* has a worldwide distribution and is commonly found in dogs causing many unwanted reactions in the host, such as alterations of the immunological response, decrease in the nutritional conversion, and predisposition to hosting various pathogenic microorganisms. The parasite's eggs are eliminated in the environment with the host's feces, surviving for several years and being a source of infection for other canids and even for human, in which they will fulfill their biological cycle (Urquhart et al., 1998; Schimmel et al., 2009).

The slow development of *T. vulpis*, together with the subclinical signs, could be the reason why there is a general belief that the *T. vulpis* infections in dogs may be less pathogenic. However, the infections can reduce growth in puppies due to the resistance to some anthelminthic drugs. In this way, alternative control measures for domestic animals' parasitoses have been the target of many researchers around the world. Among these, nematophagous fungi stand out as a viable and promising control alternative (Braga et al., 2008a, 2010; Silva et al., 2009).

Nematophagous fungi are classified into predators, endoparasites, and opportunists. These are cosmopolitan fungi, occurring in natural and agricultural soils and in all types of organic matter in decomposition (Araújo et al., 2004). In the group of predator fungi, the genera *Arthrobotrys*, *Duddingtonia*, and *Monacrosporium* stand out for their effective environmental control of nematodes by forming traps (Dimander et al., 2003; Silva et al.,

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2009). Among the opportunistic fungi, the species *Pochonia chlamydosporia* stands out (Gams and Zare, 2001). According to Lysek and Sterba (1991), the action of this fungus is based on appressorial formation, developed from undifferentiated hyphae, which allows the colonization of the egg surface and penetration through both mechanical and enzymatic actions, characterizing a type 3 effect (eggs destruction).

The objective of the present study was to evaluate the *in vitro* action of the nematophagous fungi *Duddingtonia flagrans*, *Monacrosporium thaumasium*, and *P. chlamydosporia* on *T. vulpis* eggs.

2. Materials and methods

2.1. Fungi

Four isolates of nematophagous fungi: one from de *D. flagrans* (AC001), one from *M. thaumasium* (NF34a), and two from *P. chlamydosporia* (VC1 and VC4) were kept in test tubes containing 2% corn–meal–agar, in the dark, at 4 °C for 10 days. Culture disks, 4 mm in diameter, were extracted from fungi isolates kept in the test tubes and plated into 9 cm diameter Petri dishes containing 20 mL of 2% potato–dextrose–agar, and then stored in the dark, at 25 °C for 10 days. After growth of the isolates, new culture disks, 4 mm in diameter, were transferred to 9 cm diameter Petri dishes containing 20 mL of 2% water–agar culture medium (2% WA) and for 10 days.

2.2. Obtaining of T. vulpis eggs

The eggs were recovered from the dissection of an adult female specimen obtained from the large intestines of dogs naturally infected, dead from natural causes, and further necropsied at the Veterinary Department of the Universidade Federal de Viçosa. The eggs were recovered from an adult worm. The identification of the parasite should be enough to attribute a species to the eggs. The eggs were identified according to the parameters set by Urquhart et al. (1998).

2.3. Experimental assay

The eggs were morphologically analyzed for their integrity under light microscopy (10× objectives) and plated on 9.0 cm diameter Petri dishes with 2% WA culture medium with the fungal isolates grown for 10 days and control without fungus, with 10 repetitions per group. Each plate containing one thousand *T. vulpis* eggs was assayed against one fungal isolate only. At the intervals 7, 14 and 21 days, approximately 100 eggs were removed from each plate according to Araújo et al. (1995), placed in glass slides with a drop of 1% Amam blue corant and examined under a 40× lens according to Lysek et al. (1982): type 1, lytic effect without morphological damage to eggshell, with hyphae adhered to the shell; type 2, lytic effect with morphological alteration of embryo and eggshell, without hyphal penetration through the eggshell; and type 3, lytic effect with morphological alteration of embryo and eggshell, with hyphal penetration and internal colonization.

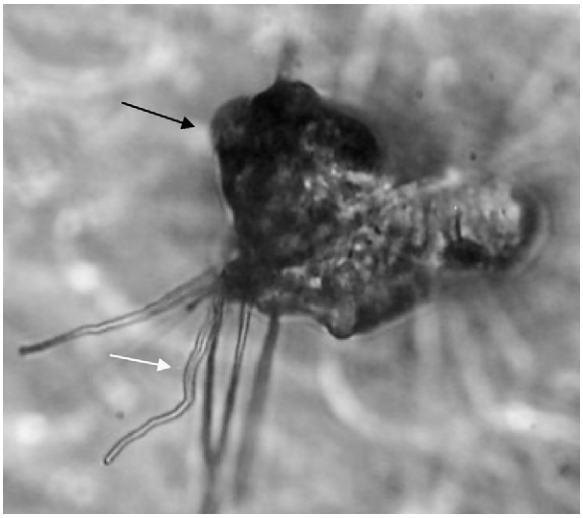

Fig. 1. Broken eggs of *Trichuris vulpis* (white arrow) and hyphae the *Pochonia chlamydosporia* (black arrow), after 21 days of interaction. Optical microscopy—40× objective lens.

2.4. Statistical analysis

Data from each studied interval were examined by non-parametric Friedman test at 5% probability (Ayres et al., 2003).

3. Results

The ovicidal activity of the fungi is shown in Table 1. Data analysis showed difference ($p < 0.05$) for ovicidal activity among the studied isolates, demonstrating that both VC1 and VC4 isolates of *P. chlamydosporia* showed type 3 effect of 29.5% and 36.5%; 59.5% and 2.5%; 94.8% and 2.9%, for the intervals 7, 14 and 21 (Fig. 1) days, respectively. The percentages found characterize *P. chlamydosporia* as an ovicidal fungus; that VC1 has a strong ovicidal activity between 14 and 21 days. However, there were no larger variations in the activity among the days of observation, reaching maximum ovicidal activity between 14 and 21 days. *T. vulpis* eggs destruction was not observed in the control group during the experimental assay (Fig. 2).

The fungi *D. flagrans* (AC001) and *M. thaumasium* (NF34a) only had type 1 effect on *T. vulpis* eggs, not showing types 2 and 3 effects, with no injury or destruction of the eggs.

4. Discussion

The fungi *D. flagrans* (AC001) and *M. thaumasium* (NF34a) did not show ovicidal activity, since Lysek (1976) only considers as ovicidal the fungi with type 3 effect on nematode eggs. In a study with trematode eggs, Braga et al. (2008a,b) registered that these fungi also showed only type 1 effect on the eggs of *Fasciola hepatica* and *Schistosoma mansoni* in the intervals 7, 14 and 21 days. Although

Table 1
Percentages and standard deviation of ovicidal activity for the nematophagous fungus *Duddingtonia flagrans* (AC001), *Monacrosporium thaumasium* (NF34a), *Pochonia chlamydosporia* (VC1 and VC4) and the control without fungal treatment, against eggs of *Trichuris vulpis* after 7, 14 and 21 days of interaction.

Isolates	Without alteration[a]	Effect type 1[b]	Effect type 2[c]	Effect type 3[d]
7 days of interaction				
AC001	51A ± 7.38	49A ± 7.38	0A ± 0	0A ± 0
NF34a	47.5A ± 11.36	52.5A ± 11.36	0A ± 0	0A ± 0
VC1	5B ± 6.24	23B ± 8.57	42.5B ± 10.07	29.5B ± 9.27
VC4	9.5B ± 4.38	18.5B ± 6.69	36B ± 9.37	36.5B ± 36.5
Control	10C ± 0	0C ± 0	0C ± 0	0C ± 0
14 days of interaction				
AC001	42A ± 12.06	57A ± 10.85	0A ± 0	0A ± 0
NF34a	1.2AB ± 1.03	98.7AB ± 0.95	0A ± 0	0A ± 0
VC1	0.5B ± 1.58	55A ± 8.64	35.5B ± 16.74	59.5B ± 22.79
VC4	2B ± 6.33	5.5A ± 9.56	19.5B ± 9.85	2.5B ± 73
Control	10C ± 0	0C ± 0	0C ± 0	0C ± 0
21 days of interaction				
AC001	33A ± 5.87	67A ± 5.87	0A ± 0	0A ± 0
NF34a	1.1A ± 0.74	98.9A ± 0.74	0A ± 0	0A ± 0
VC1	0B ± 0	0.8AB ± 1.62	4.4B ± 3.13	94.8B ± 4.1
VC4	0B ± 6.68	0.2B ± 10.42	4.1B ± 5.8	2.95B ± 95.7
Control	10C ± 0	0C ± 0	0C ± 0	0 ± 0

Percentages followed by same letter (A, B, C) in the same column are not significantly different ($p > 0.05$)—Friedman test.
[a] Without alteration.
[b] Effect type 1, lytic effect without morphological damage to eggshell, with hyphae adhered to the shell.
[c] Effect type 2, lytic effect with morphological alteration of embryo and eggshell, without hyphal penetration through the eggshell.
[d] Effect type 3, lytic effect with morphological alteration of embryo and eggshell, with hyphal penetration and internal colonization.

D. flagrans and *M. thaumasium* have been recognized as biocontrol agents of nematode larvae, in this work, hyphae of both fungi that were adhered to the eggshell did not cause egg destruction. A viability test should comprehend the administration of treated eggs to susceptible animals that have been previously in contact with the fungi (Morgan-Jones and White, 1983).

The first mode of mechanical action mentioned for an ovicidal fungus is the appressorium, which is the structure used for egg penetration (Lysek, 1978). Besides, according to Stirling and West (1991), the direct effect of fungal parasitism on embryo development is through the enzymatic action on the eggshell by increasing permeability and facilitating the passage of toxins.

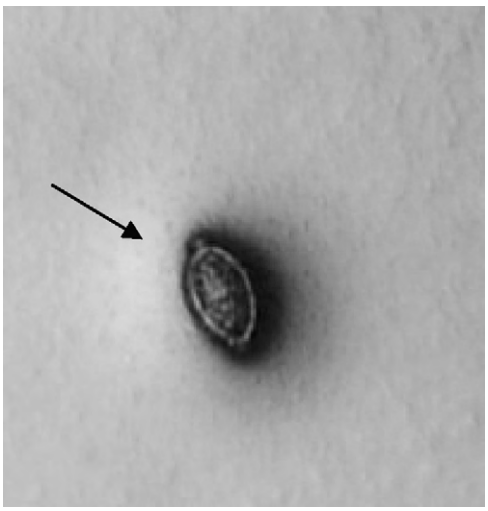

Fig. 2. Show *Trichuris vulpis* of the control group, without fungus.

In the present work, these isolates had greater percentages of ovicidal activity on *T. vulpis* eggs compared with the reports by Braga et al. (2007) with eggs of *A. lumbricoides*. The results obtained with *A. lumbricoides* with the isolates VC1 and VC4 were more homogeneous, even if the ovicidal activity was lower compared with *D. flagrans* and *M. sinense* in the intervals 7, 10 and 14 days of interaction, observing that isolates VC1 and VC4 showed type 3 effect of 20% and 18%; 25% and 22%; and 30% and 26%, respectively. These results indicate there are differences in the interaction process between the isolates and the eggs of the studied nematodes. In this way, further research is necessary for greater elucidation of this fact. *P. chlamydosporia* has been successfully tested on eggs of various genera of gastrointestinal parasite helminths (Braga et al., 2007, 2010; Araújo et al., 2008). However, the present work is the first report on ovicidal activity on *T. vulpis* eggs.

5. Conclusion

The results of this work indicate that the *P. chlamydosporia* was effective in destroying *in vitro T. vulpis* eggs and may contribute to decrease environmental contamination by this nematode's eggs.

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