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Interaction and ovicidal activity of nematophagous fungus *Pochonia chlamydosporia* on *Taenia saginata* eggs

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ABSTRACT

The ovicidal activity of the nematophagous fungi *Pochonia chlamydosporia* (isolates VC1 and VC4), *Duddingtonia flagrans* (isolate AC001) and *Monacrosporium thaumasium* (isolate NF34) on *Taenia saginata* eggs was evaluated under laboratory conditions. *T. saginata* eggs were plated on 2% water-agar with fungal isolates and controls without fungus and examined after 5, 10 and 15 days. At the end of the experiment *P. chlamydosporia* showed ovicidal activity against *T. saginata* eggs ($p < 0.05$), mainly for internal egg colonization with results of 12.8% (VC1) and 2.2% (VC4); 18.1% (VC1) and 7.0% (VC4); 9.76% (VC1) and 8.0% (VC4) at 5, 10 and 15 days, respectively. The other fungi showed only lytic effect without morphological damage to the eggshell. Results demonstrated that *P. chlamydosporia* was effective *in vitro* against *T. saginata* eggs unlike the other fungi.

1. Introduction

Helminthosis remains an important problem for public health (Jongwutiwes et al., 2004). In humans, infections by cestodes of the taeniasis/cysticercosis complex affect up to 77 million people. The *Taenia saginata* has a cosmopolitan distribution and is found widely in most countries that raise cattle. Brazil occupies an important position globally as the largest commercial producer (Alves, 2001). There are no systematic or reliable data available to determine the prevalence of this disease in humans and animals (Pereira et al., 2006). The specialized literature, however, suggests that some groups of people may be at greater risk because of economic, cultural or even religious factors (Pfuetzenreiter and Ávila-Pires, 2000). The occurrence of infection is directly related to poor sanitary conditions and socioeconomic characteristics of the population (González et al., 2002). The life cycle of *T. saginata* includes a definitive host, an intermediate host and a free-living phase. Adult worms live in the small intestine of humans, the only definitive host. Intermediate hosts of are cattle, where the parasite develops in voluntary muscles (Acha and Szyfres, 1986). Fecal contamination of soil is considered as one of the factors that can favor egg dispersion in the environment (Gemmell and Lawson, 1982). A number of preventive measures can be applied to control the spread of infection, but the main strategy consists of interrupting the parasite life cycle (Organizacion Panamericana de la Salud, 1994). Geohelminths are cosmopolitan parasites that depend on multiple factors to stay in the environment and complete their life cycle. Soil containing infective geohelminth eggs is the main source of infection both in humans and animals. Antagonists, such as ovicidal fungi can influence the development and presence of eggs in the soil and cause their destruction (Lysek et al., 1982; Morgan-Jones and Rodriguez-Kabana, 1985). Studies on natural processes of destruction of geohelminth eggs are still in the initial stages, but they represent an alternative approach which, if used with other prophylactic measures, may help control species of epidemiological importance. Nematophagous fungi are divided into predators, endoparasites and opportunists; *Pochonia chlamydosporia* is ovicidal whereas *Monacrosporium thaumasium* and *Duddingtonia flagrans* are predators. Nematophagous fungi are widely distributed and can be found in a great diversity of species as well as in different ecosystems (Gray, 1983). These species have proven effectiveness against eggs and infective larvae of gastrointestinal helminth parasites of domestic animals. (Braga et al., 2008a; Araújo et al., 2007, 2008; Mota et al., 2003). Following a prolonged observational study, Lysek (1976) established a qualitative method for classifying ovicidal activity. This method, first proposed for the activity of *Pochonia chlamydosporia* against *A. lumbricoides* eggs, determines that the mechanism of action of an ovicidal fungus is based on three basic

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types of activity and seven subtypes, as follows: (1) physiological; biochemical effect without morphological damage to eggshell; (2) lytic biochemical effect, with progressive morphological, changes of the eggshell and damage to the embryo; and (3) lytic and morphological effect, with hyphal penetration of eggs, attack and death of the embryo; and the subdivisions: (Lysek,1976; Lysek et al., 1982; Lysek and Nigenda, 1989; Lysek and Sterba, 1991).

Fungal predators vary in their ability to capture gastrointestinal helminth parasites using traps. The genera *Monacrosporium* and *Duddingtonia* stand out in this group (Araújo et al., 2004; Braga et al., 2007).

This study aimed at evaluating the ovicidal effect of the nematophagous fungi *P. chlamydosporia, M. thaumasium* and *D. flagrans* on eggs of *T. saginata*, a potential pathogen for humans.

2. Materials and methods

2.1. Organisms

Four isolates of nematophagous fungi; two *P. chlamydosporia* (VC1 and VC4); one *D. flagrans* (AC001) and one *M. thaumasium* (NF34) were used in the study. These were originally obtained from soil collected in the municipality of Viçosa, Minas Gerais, Brazil, 20°45′20" latitude and 42°52′40" longitude, using the sprinkling method (Duddington, 1955), modified by (Santos et al., 1991), and kept at the Parasitology Laboratory in the Veterinary Department of the Federal University of Viçosa, MG.

2.2. Taenia saginata eggs

T. saginata eggs were morphologically analyzed for integrity under an optical microscope using a 10× objective lens. The eggs were recovered by dissection of adult proglottids obtained from human feces. Proglottid identification followed criteria established by Urquhart et al. (1998) and Bowman et al. (2006).

2.3. Experimental essay

The four isolates were kept in test tubes containing 2% corn-meal-agar (2% CMA), in the dark, at 4 °C for 10 days. Culture disks, 4 mm in diameter, were removed from grown fungal colonies, plated onto 9-cm diameter Petri dishes containing 20 mL of 2% water-agar (2% AA) and stored in the dark, at 25 °C for 10 days. Fungal growth covered the whole of the surface after 10 days. One thousand *T. saginata* eggs were placed on the surface of 9.0-cm Petri dishes containing 2% AA and the fungal isolates and a control without fungi, with 25 replicates per group. After 5, 10 and 15 days, 100 eggs were removed from the plates treated with *P. chlamydosporia* (VC1 and VC4), *D. flagrans* (AC001) and *M thaumasium* (NF34) and from each control plate (without fungi) as described by Araújo et al. (1995): using a spatula, the eggs were placed on the surface of slides containing a drop of 1% Amam-blue. Eggs were examined under a light microscope (40× objective lens) and scanning electron microscope and evaluated according to Lysek et al. (1982): type 1 effect, physiological and biochemical effect without morphological damage to eggshell, with visualization of hyphae adhered to eggshell; type 2 effect, lytic effect with morphological changes in embryo and eggshell, without hyphal penetration through the eggshell, and type 3, lytic effect with morphological changes of embryo and eggshell, with hyphal penetration and internal egg colonization. Data from each time were analyzed by Friedman's nonparametric test at 5% probability.

3. Results and discussion

Results for type 1, 2 and 3 effects at 5, 10 and 15 days for *P. chlamydosporia* (VC1 and VC4), *M. thaumasium* (NF34) and *D. flagrans* (AC001) are shown in Table 1–3. Isolate VC1 showed type 1, 2 and 3 effects at all three times. Isolate VC4 showed slightly less effect at all time points.

Examination by optical and scanning electron microscopy showed *P. chlamydosporia* hyphae on egg surfaces (Fig. 1a and b)

Table 1
Percentage and standard deviation (±) for effects types 1*, 2** and 3*** of the nematophagous fungi *Pochonia chlamydosporia* (VC1 and VC4), *Monacrosporium thaumasium* (NF34) and *Duddingtonia flagrans* (AC001) on *Taenia saginata* eggs on the 5th day of interaction.

Isolates	Effect at five day		
	Effect type 1*	Effect type 2**	Effect type 3***
AC001	$65.0^{A} \pm 15.3$	$0^{B} \pm 0$	$0^{B} \pm 0$
NF34	$72.4^{A} \pm 16.0$	$0^{B} \pm 0$	$0^{B} \pm 0$
VC1	$23.9^{B} \pm 6.3$	$21.7^{A} \pm 17.9$	$12.8^{A} \pm 11.6$
VC4	$18.4^{B} \pm 6.0$	$9.9^{A} \pm 5.4$	$2.3^{C} \pm 1.9$
Control	$0^{C} \pm 0$	$0^{B} \pm 0$	$0^{B} \pm 0$

* Effect type 1, physiological and biochemical effect without morphological damage to eggshell, with visualization of hyphae adhered to eggshell.
** Effect type 2, lytic effect with morphological changes in embryo and eggshell, without hyphal penetration through the eggshell.
*** Effect type 3, lytic effect with morphological changes of embryo and eggshell, with hyphal penetration and internal egg colonization. Percentages followed by identical letters (A, B, C) in the same column do not differ ($p > 0.05$) – Test of Friedman.

Table 2
Percentage and standard deviation (±) for effects types 1*, 2** and 3*** of the nematophagous fungi *Pochonia chlamydosporia* (VC1 and VC4), *Monacrosporium thaumasium* (NF34) and *Duddingtonia flagrans* (AC001) on *Taenia saginata* eggs on the 10th day of interaction.

Isolates	Effect at 10 days		
	Effect type 1*	Effect type 2**	Effect type 3***
AC 001	$68.1^{A} \pm 15.0$	$0^{B} \pm 0$	$0^{B} \pm 0$
NF34	$74.4^{A} \pm 15.9$	$0^{B} \pm 0$	$0^{B} \pm 0$
VC 1	$24.0^{B} \pm 8.3$	$24.2^{A} \pm 8.5$	$18.2^{A} \pm 9.3$
VC 4	$17.5^{B} \pm 4.9$	$12.3^{C} \pm 5.4$	$7.0^{C} \pm 3.8$
Control	$0^{C} \pm 0$	$0^{B} \pm 0$	$0^{B} \pm 0$

* Effect type 1, physiological and biochemical effect without morphological damage to eggshell, with visualization of hyphae adhered to eggshell.
** Effect type 2, lytic effect with morphological changes in embryo and eggshell, without hyphal penetration through the eggshell.
*** Effect type 3, lytic effect with morphological changes of embryo and eggshell, with hyphal penetration and internal egg colonization. Percentages followed by identical letters (A, B, C) in the same column do not differ ($p > 0.05$) – Test of Friedman.

Table 3
Percentage and standard deviation (±) for effects types 1*, 2** and 3*** of the nematophagous fungi *Pochonia chlamydosporia* (VC1 and VC4), *Monacrosporium thaumasium* (NF34) and *Duddingtonia flagrans* (AC001) on *Taenia saginata* eggs on the 15th day of interaction.

Isolates	Effect at 15 days		
	Effect type 1*	Effect type 2**	Effect type 3***
AC001	$72.6^{A} \pm 16.3$	$0^{B} \pm 0$	$0^{B} \pm 0$
NF34	$77.3^{A} \pm 17.2$	$0^{B} \pm 0$	$0^{B} \pm 0$
VC1	$22.3^{B} \pm 8.4$	$22.2^{A} \pm 8.4$	$9.7^{A} \pm 5.7$
VC4	$17.2^{B} \pm 5.7$	$17.2^{C} \pm 6.3$	$8.0^{A} \pm 4.0$
Control	$0^{C} \pm 0$	$0^{B} \pm 0$	$0^{B} \pm 0$

* Effect type 1, physiological and biochemical effect without morphological damage to eggshell, with visualization of hyphae adhered to eggshell.
** Effect type 2, lytic effect with morphological changes in embryo and eggshell, without hyphal penetration through the eggshell.
*** Effect type 3, lytic effect with morphological changes of embryo and eggshell, with hyphal penetration and internal egg colonization. Percentages followed by identical letters (A, B, C) in the same column do not differ ($p > 0.05$) – Test of Friedman.