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Research Note

Biological control of *Taenia saginata* eggs

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Summary

Taenia saginata, is a cestode with zoonotic potentially. The objective of this work was to evaluate the ovicide activity (type 3 effect) of the fungus, *Paecilomyces lilacinus* (PL1), on the eggs of *Taenia saginata, in vitro.* The eggs were inverted in Petri dishes containing PL1, or in Petri dishes without the fungus (control). After 5, 10, or 15 days, we extracted approximately 100 eggs for evaluation and for classifying the ovicide activity. At the end of the 15 days of interaction, a significant difference ($p < 0.05$) due to the ovicide activity of PL1, was noted in relation to the control group, with a medium percentage (type 3 effect) at 24.6 %. These results show that the fungus, *P.lilacinus* (PL1), destroyed the eggs of *T. saginata,* suggesting its biological control potential of the eggs of this cestode.

Keywords: Nematophagous fungus; *Paecilomyces lilacinus*; zoonosis; *Taenia saginata*

Introduction

According to Carvalho *et al*. (2010), helminthic infections pose a public health problem, within the same context, the taeniasis-cysticercosis complex affecting around 45 million people worldwide with Brazil being in focus, being the world largest beef producer (Lateef *et al*., 2007). The life cycle of *T. saginata involves two hosts:* one definite (man) in the small intestines and the other being intermediate (cattle) (Acha & Szifre, 1986). Three phases referring to its metamorphosis are known: the first being the adult found in man, the environmental phase (that is the infective eggs found in the soil) and larvae found in the intermediate host (Acha & Szifre, 1986). Various strategies are employed in combating the taeniasis-cysticercosis complex, though the main being interrupting the parasites' biological evolution cycle (Organización Panamericana de La Salud, 1994). In this context, it means using nematophagic fungi controlling biologically infective eggs in the soil, thus controlling the development, permanence and subsequently their destruction (Carvalho *et al*., 2010; Frassy *et al*., 2010). Nematophagic fungi could be predators, endoparasites or ovicidic in relation to the eggs of *Taenia saginata.* Note that they naturally reside in the soil, as well as various decomposing matter (Braga *et al*., 2010). On the other hand, fungal parasites with saprophytic characteristics are easily cultivable in the laboratory and commercially employed, due to the fact that they colonize the egg contents leading to their destruction. In controlling various genera of gastro-intestinal helminthic parasites, ovicide fungi like *Paecilomyces lilacinus* and *Pochonia chlamydosporia* have been used both in lab as well as out-door environments with success (Braga *et al*., 2010; Singh *et al*., 2010). Contrasting though, little literature has highlighted the importance of *P. lilacinus* in controlling geo-helminthes' eggs, principally those of *T. saginata*, a potential zoonotic cestode (Carvalho *et al*., 2010).

This work evaluates the ovicide capacities (type 3 effect) of the fungus *Paecilomyces lilacinus* (PL1) on the eggs of *Taenia saginata* in laboratory conditions.

Materials and Methods

Fungus

We employed an isolate of the nematophagic fungus *Pae-*

cilomyces lilacinus (PL1). This species is a native of Brazil, within the locality of Viçosa, in the state of Minas Gerais latitude 20°45'20"S, longitude 42°52'40" W, at 649 m altitude. The fungus was maintained in test tubes containing a corn-meal-agar 2 % solution at 4º C, kept in the dark, lasting 10 days. After the growth of our isolate, we transferred cultured disks measuring 4.0 mm diameter to Petri dishes measuring 9.0 cm diameter and containing 20 mL of water-agar- 2 % solution staying at this condition for a further 10 days.

Obtaining the eggs of Taenia saginata and the experimental model

The eggs of *T. saginata* were derived from the proglottis section of an adult sample, voluntarily handed over by a patient with taeniasis diagnosis. On following, they were analyzed microscopically (light microscope at objective 10X, Urquhart *et al*., 1998) checking on their integrity and viability. Consequently, approved eggs were washed 10 times in centrifuged distilled water (1000 X g), each round lasting 5 minutes with the supernatant discarded each time. For 14 days, we incubated the eggs at 25º C in a solution consisting of Phormol 0.05 %, added to 0.005 % Streptomycine Sulphate and Chloraphenicol 0.01 %, as described by Carvalho *et al*., (2010).

The eggs of *T. saginata* were verted over a Petri dish, 9.0 cm diameter, containing 2 % WA with the fungi isolates grown 10 days and in another setup without the fungal sample (control) but containing only 2 % WA, each procedure repeated 25 times. In the end, each dish had a total of 1000 eggs. During the 5, 10 and the 15 day interval, we fished out approximately 100 eggs from each dish as des-cribed (Braga *et al*., 2010). Next, we evaluated our samples as established by Lysek *et al*. (1982): type 1 (physiological effect with no significant change noted on the egg-shell, despite the hyphi adhering to the respective shell), type 2 (lytic effect where morphological alterations on the egg-shell and the embryo are present, without the hyphi penetrating the shell) and type 3 (lytic effect accompanied with morphological changes on the egg-shell and the embryo, hyphal penetration and interior egg-colonization is present. Data derived at each interval was studied and submitted for analysis to non-paired Friedman-test with 5 % probability. Our data analysis was auxiliated with Biostat 3.0 software (Ayres *et al*., 2003).

Results and Discussion

The results of each procedural effect by *P. lilacinus* (PL1) on the eggs of *T. saginata* are presented according to each respective interval (5, 10 and 15 day-interval) in percentage form - Table 1. Statistical analysis revealed a significant difference ($p < 0.05$) at each interval, with reference to the ovicide activity of PL1 on the eggs of *T. saginata* in the control group; thus, proving its ovicide potential. When comparing one studied treated group to another during the 5, 10 and 15 day-interval, no significant change was noted ($p < 0.01$): fact that the ovicide activity was on course during the first day of observation (5th day). The type 3 effect (characterized by the destruction of the eggs) due to the effect of *P. lilacinus* (PL1) over the three intervals were respectively: 23.8 % (5th day), 25.4 % (10th day) and 24.8 % (15th day). Besides, observation under a light microscope 40X, proved the ovicide activity (type 3) of *P. lilacinus* over the eggs of *T. saginata.*

According to Braga *et al*. (2010), the principle effect of an ovicide fungus is the type 3 role in course of infestation. This characteristic was manifested by the isolate of PL1 on the eggs of *T. saginata*, suggesting that its biological use for controlling taenia proliferation and infection is promising. Note that type 1 and type 2 effects demonstrated by data do not classify PL1 as ovicide. This agrees with literature reporting that the aforementioned effects, though not proving ovicide, do contribute to biological control due to their viability-reducing capacities (Araújo *et al*., 2009). This fact has been earlier noted in other protocol-models involving genera *Duddingtonia,* and *Monacrosporium* and their effect on gastrointestinal infesting helminthic parasites (Silva *et al*., 2010, Carvalho *et al*., 2010). Little literature address on the importance of biological methods of controlling cestode infections and proliferation, which could otherwise help in evading future natural reinfection episodes. Braga *et al*. (2008), demonstrated that *P. lilacinus* posted a significant efficiency index ($p < 0.01$) *in vitro* on the eggs of *Moniezia* spp., a cestode in relation to a control model set up, thus characterizing it as ovicidal (27.0 %, of type 3 effect at the end of study). The results reported by the authors are in line with those posted in our present work.

No fungi species distinct themselves due their ovicide nature more than *P. lilacinus* and *P. chlamydosporia* (Braga *et al.,* 2010). Araújo *et al.* (2009) reported that *P. chlamydosporia* (VC1 e VC4) samples destroyed eggs of *T. saginata in vitro* at the end of 15 days. When comparing to the efficiency of *P. lilacinus* in our work, it can be noted that: (1) in relation to type 1 effect, both did not post significant difference ($p > 0.05$). (2) in relation to type 2 effect, PL1-isolate posted a significant difference ($p < 0.05$) in comparison to VC4-isolate in all observed day-intervals, with greater efficacy. On the other hand, there was no significant difference noted ($p > 0.05$) between PL1-isolate and VC1-isolate. (3) in relation to type 3 effect, PL1-isolate posted a significant difference ($p < 0.05$) when compared to the VC1-isolate at the end of the 15 days of fungi-egg interaction. When compared to the VC4-isolate, PL1 showed a statistical difference ($p < 0.05$) in the course of the 15 days under study (type 3 effect).

The inference aforementioned show that *P. lilacinus* is a promissing cestode biological controller, in line with what other authors referred to biological cestode control by *P. lilacinus* (Braga *et al*., 2008). On the other hand, this fungus is efficient in destroying the eggs of nematodes (Braga *et al*., 2010). Eggs of *T. saginata* are resistant to environment adverse condition, conventional sewerage treatment besides remaining viable up to 12 months due to the fact them having a hard shell protective later, embryophore

Table 1. Percentage and standard deviation values of the ovicide activity (type 1, 2 and 3) of the nematophagic fungus *Paecilomyces lilacinus* (PL1) in relation to the control group (without the fungus) on the eggs of *T. saginata* during the day 5, 10 and day 15-intervals of fungus-egg interaction.

Groups	**Effect at 5 days**		
	Effect Type 1^{+}	**Effect Type 2^{++}**	**Effect Type 3^{+++}**
Paecilomyces lilacinus	$24.8^{A} \pm 10.8$	$26.5^{A} \pm 9.5$	$23.8^{A} \pm 11.8$
Control	$0^{B} \pm 0$	$0^{B} \pm 0$	$0^{B} \pm 0$
Groups	Effect at 10 days		
Paecilomyces lilacinus	$24.2^{A} \pm 7.8$	$25.5^{A} \pm 7.8$	$25.4^{A} \pm 8.1$
Control	$0^{B} \pm 0$	$0^{B} \pm 0$	$0^{B} \pm 0$
Groups	Effect at 15 days		
Paecilomyces lilacinus	$25.3^{A} \pm 11.2$	$29.0^{A} \pm 8.7$	$24.8^{A} \pm 8.0$
Control	$0^{B} \pm 0$	$0^{B} \pm 0$	$0^{B} \pm 0$

Percentages followed by same letter in the same column are not significantly different (P>0.01) – Friedman test.
Physiological, biochemical effect without morphological damage to eggshell, with hyphae adhered to the shell
**Lytic effect with morphological alteration of embryo and eggshell, without hyphal penetration through the eggshell.
***Lytic effect with morphological alteration of embryo and eggshell, besides hyphal penetration and internal colonization

(Urquhart *et al*., 1998). This provokes a need of using alternative ways of combating its environmental proliferation and subsequent human-animal infection, in this case biological means using ovicidal fungi with potential of propagating in fecal matter-conditions (Araújo *et al*., 2009). However the mechanism of ovicide fungal egg shell penetration is yet to be totally elucidated. But it is believed that physical, mechanical followed by enzymatic activity are some of the components involving egg colonization and penetration (Carvalho *et al*., 2010). In his recent work, Singh *et al*. (2010) mentions that biological and anti-helminthes ways could be employed in controlling animal infestation with success during the raring course. In this work, the efficacy of the fungus *P. lilacinus* (PL1) in destroying the eggs of *T. saginata* in vitro, was noted suggesting that it as a biological tool in controlling egg-proliferation of this cestode is justified.

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