Activity of nematophagous fungi *Pochonia chlamydosporia* and *Paecilomyces lilacinus* on *Dipylidium caninum* egg capsules

Atividade dos fungos nematófagos *Pochonia chlamydosporia* e *Paecilomyces lilacinus* sobre cápsulas de ovos de *Dipylidium caninum*

RIALA6/1248

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Recebido: 03.09.2008 – Aceito para publicação: 07.12. 2009

ABSTRACT
Nematophagous fungi are potential agents to be employed for biological control of helminthes. The ovicidal activity of the nematophagous fungi *Pochonia chlamydosporia* (isolates VC1 and VC4) and *Paecilomyces lilacinus* on egg capsules of *Dipylidium caninum,* a cestoda parasite of dogs, cats and men, was evaluated on Petri dishes cultures. One thousand of *D. caninum* egg capsules were placed onto Petri dishes containing 2% water-agar medium and grown fungal isolates, and also onto dishes without fungi, as control. The ovicidal activity of these fungi was evaluated after 5, 10 and 15 days. After the beginning of the interaction and at the end of the experiment, fungi *P. chlamydosporia* and *Paecilomyces lilacinus* demonstrated ovicidal activity ($p<0.05$) when compared to the control. *Pochonia. chlamydosporia* showed ovicidal activity of 49.0% (isolate VC1) and 41.9% (isolate VC4), while ovicidal activity of *Paecilomyces lilacinus* was 42.7% after fifteen days of interaction. The fungi *Pochonia chlamydosporia* and *Paecilomyces lilacinus* showed ovicidal activity on *Dipylidium caninum* egg capsules, thus it could be used as potential biological controllers of this cestoda.
Key words. Nematophagous fungi, *Pochonia chlamydosporia, Paecilomyces lilacinus, Dipylidium caninum,* Biological control.

RESUMO
Os fungos nematófagos são potenciais agentes empregados no controle biológico de helmintos. A atividade ovicida dos fungos nematófagos *Pochonia chlamydosporia* (isolados VC1 e VC4) e *Paecilomyces lilacinus* sobre cápsulas ovígeras de *Dipylidium caninum*, foi avaliada em culturas em placas de Petri. Mil cápsulas ovígeras de *D. caninum* foram colocadas em placas de Petri contendo 2% do meio ágar-água e isolados fúngicos cultivados e também em placas sem fungo, como controle. A atividade ovicida desses fungos foi avaliada após cinco, 10 e 15 dias. Após o começo da interação e ao final do experimento, os fungos *P. chlamydosporia* e *Paecilomyces lilacinus* demonstraram atividade ovicida ($p< 0,005$) quando comparados ao controle. *Pochonia. chlamydosporia* demonstrou atividade ovicida de 49.0% (isolado VC1) e 41.9% (isolado VC4), e a atividade ovicida do *Paecilomyces lilacinus* foi de 42.7% ; após 15 dias de interação. Os fungos *Pochonia chlamydosporia* e *Paecilomyces lilacinus* apresentaram atividade ovicida sobre cápsulas ovígeras de *Dipylidium caninum* , o que indica a viabilidade de efetuar o seu emprego como potencial controlador biológico desse cestoda.
Palavras chaves. fungos nematófagos, *Pochonia chlamydosporia, Paecilomyces lilacinus, Dipylidium caninum,* Controle biológico.

According to the World Health Organization (WHO), about 2 billion people infected by zoonoses live in underdeveloped countries. 300 million people are seriously ill, and 50% of them are children at school age. In developing countries, verminosis represents one of the main causes of epilepsy in children[1]. Lack of sanitation is one of the main reasons for the increase in the occurrence of zoonoses which endemically attack the Third World. Helminths are endemic cosmopolitan parasites whose permanence in the contaminated environment depends on several factors that allow their evolution cycle to develop. *Dipylidium caninum* is among those parasites. It is a cestoda that belongs to the family Dilepididae, a gastrointestinal parasite of canidae and felidae, which sometimes parasites human beings, especially children at school age [1, 2].

According to Pereira et al.[1] close contact with animals that live in endemic areas and unhygienic conditions, as regards both human and animal health are also great predisponent factors for the occurrence of zoonoses. The contamination of the definitive host (dogs, cats and, accidentally, humans) is caused by the ingestion of infected intermediate hosts (fleas and lice)[3].

The fungus species *Pochonia chlamydosporia* and *Paecilomyces lilacinus* are considered ovicides and have proven efficiency on eggs of gastrointestinal parasite helminths in domestic animals and human beings[4, 5]. Therefore, the use of these fungi might help in the control of egg capsules and eggs of *D. caninum* that are present in the environment.

The objective of this study was to evaluate the activity of nematophagous fungi of the species *P. chlamydosporia* and *P. lilacinus* on egg capsules of *D. caninum.*

The fungi *P. chlamydosporia* (VC1 and VC4) and *P. lilacinus* were stored in test tubes at 4ºC containing 2% corn-meal-agar and maintained, in the dark, for 10 days. After their growth, new culture discs (4mm in diameter) were transferred to Petri dishes (9cm in diameter) containing 20mL of 2% water-agar (2%WA) for 10 days.

The egg capsules of *D. caninum* were recovered from the dissection of proglottids of an adult individual and morphologically evaluated as to their integrity in optical microscope with 10x objective lens.

The egg capsules were placed on the surface of Petri dishes (9cm in diameter) containing the 2%WA medium, with fungus free fungal isolates grown for 10 days, as control. Twenty-five replications were performed for each group. In the treatments, each dish contained one thousand egg capsules of *D. caninum* with only one of the fungal isolates. In the intervals of 5, 10 and 15 days, approximately one hundred egg capsules were removed from each dish containing the fungus and from those containing the control (fungus free), according to the technique described by Araújo et al.[6]. They were evaluated with 40x objective lens, according to the parameters established by Lysek et al.[7]: type 1, lytic effect without morphological damage to the eggshell, with hyphae adhered to the eggshell; type 2, lytic effect with morphological change of embryo and eggshell, without hyphal penetration through eggshell; and type 3, lytic effect with morphological change of embryo and eggshell, besides hyphal penetration and internal egg colonization. The data of the studied interval were submitted to the non-parametric Friedman test, with 5% of probability.

Table 1 shows the percentage results of types 1, 2 and 3 effects presented by the fungi *P. chlamydosporia* (VC1 and VC4) and *P. lilacinus*, over the experimental assay of 5, 10 and 15 days. The isolate VC1 presented the following percentages for type 1 effect: (23.0%, 5% and 10.7%); type 2 effect (19.6%, 32.9% and 32.3%); and for type 3 effect (19.6%, 44.2% and 49.2%) after 5, 10 and 15 days, respectively. The isolate VC4 presented the following percentage results (18.4%, 15.7% and 11.5%) for type 1 effect; (28.4%, 30.3% and 35%) for type 2 effect; and (20%, 31.5% and 41.9%) for type 3 effect, over the days studied. According to Lysek et al.[7], the type 3 effect classifies the fungus as a potential ovicide. In the present work, the isolates VC1 and VC4 presented this type of effect, but with no difference (P>0.05) between them. Similarly, the fungus *P.lilacinus* presented the percentages (9.9%, 9.4% and 6.4%) for type 1 effect; (11.7%, 10.4% and 9.9%) for type 2 effect, and (28.4%, 37.1% and 42.7%) for type 3 effect after 5, 10 and 15 days, respectively. Therefore, it was also considered an ovicidal fungus [5, 6, 8].

No difference (P>0.05) was observed between fungi *P. chlamydosporia* and *P. lilacinus* after their ovicidal activity was compared. It suggests that both fungi could be used in the biological control of the capsules of *D. caninum*, since they presented very similar percentage results for type 3 effect [9, 10, 11].

The results of the present study can be compared to the works of Araújo et al.[9] and Braga et al.[4, 10, 11] on eggs of *A*scaris, *Fasciola* and *Schistosoma*, corroborating the activity potential of these isolates on eggs of several

Table 1. Percentages and standard deviations of the ovicidal activity (types 1, 2 and 3 effects) of the fungi *Pochonia chlamydosporia* (VC1 and VC4) and *Paecilomyces lilacinus* and the fungus-free control group on the egg capsules of *Dipylidium caninum* after 5, 10 and 15 days of interaction.

Isolates	Effect after 5 days		
	Type 1 effect*	**Type 2 effect ****	**Type 3 effect *****
VC1	23.0 ^{B}± 33.5	19.6 ^{AB}± 23.7	19.6 ^{A}± 25.6
VC4	18.4 ^{B}± 24.1	28.4 ^{A}± 23.8	20.0 ^{A}± 26.6
P. lilacinus	9.9 ^{B}± 10.6	11.7 ^{B}± 13.7	28.4 ^{A}± 18.2
Control	0 ^{C}± 0	0 ^{C}± 0	0 ^{B}± 0
Isolates	Effect after 10 days		
	Type 1 effect *	**Type 2 effect ****	**Type 3 effect *****
VC 1	5.0 ^{B}± 16.5	32.9 ^{A}± 36.6	44.2 ^{A}± 41.2
VC 4	15.7 ^{C}± 22.8	30.3 ^{A}± 26.6	31.5 ^{A}± 32.0
P. lilacinus	9.4 ^{BC}± 12.8	10.4 ^{B}± 12.9	37.1 ^{A}± 19.5
Control	0 ^{D}± 0	0 ^{C}± 0	0 ^{B}± 0
Isolates	Effect after 15 days		
	Type 1 effect *	**Type 2 effect ****	**Type 3 effect *****
VC1	10.7 ^{B}± 20.3	32.3 ^{A}± 26.0	49.2 ^{A}± 36.5
VC4	11.5 ^{B}± 21.4	35.0 ^{A}± 26.6	41.9 ^{A}± 36.4
P. lilacinus	6.4 ^{B}± 9.5	9.9 ^{B}± 12.8	42.7 ^{A}± 32.8
Control	0 ^{C}± 0	0 ^{C}± 0	0 ^{B}± 0

Percentages followed by the same capital letter in the same column do not differ statistically (P>0.05) – Friedman test. *Physiological and biochemical effect, without morphological damage to the egg shell, where the hyphae are observed to be adhered to the shell.**Lytic effect with morphological change of the egg shell and the embryo, without hyphal penetration through the shell. ***Lytic effect with morphological change of the shell and the embryo, besides hyphal penetration and internal colonization of the egg.

helminth genera. These fungi, therefore, can be classified as ovicides.

The activity capacity of the fungus *P. lilacinus* was demonstrated in the works of Araújo et al.[6] on eggs of *Toxocara canis*, for types 1, 2 and 3 effects, respectively. In the work of Braga et al. (2008c), the authors recorded that this fungus presented percentages of type 3 effect of 19%, 20% and 23% on eggs of *Moniezia* sp after 5, 10 and 15 days, respectively.

Pereira et al.[1] carried out a study in 2005 with children ranging in age from 4 months to 14 years old in the city of Campos dos Goytacazes, in the state of Rio de Janeiro. The authors observed egg capsules of *D. caninum* in the feces of children at school age and believe the children were probably contaminated through the contact with local companion animals, thus ingesting fleas from such animals. Therefore, since the source of infection of *D. caninum* for the intermediate host (fleas) is an environment contaminated with feces, the use of the fungi *P. chlamydosporia* (VC1 and VC4) and *P. lilacinus* is suggested for the biological control of this cestoda because its action could be concentrated in the fecal environment.

The fungi *P.chlamydosporia* and *P.lilacinus* presented ovicidal activity on the egg capsules of *Dipylidium caninum* and could be used as potential biological controllers of this cestoda.

Acknowledgements

The authors thank FAPEMIG, CAPES / FINEP and CNPq for financial support.

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