Nematicide activity of microfungi (Orbiliales, Orbiliaceae) after transit through gastrointenstinal tract of "Gallus gallus domesticus"

Atividade nematiciada de microfungos ("Orbiliales, Orbiliaceae") após trânsito gastrointestinal de Gallus gallus domesticus

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SUMMARY

Parasites are common in intensive or organics systems destined for chickens, which is more conducive to the emergence of gastrointestinal parasites, favored by direct contact with soil and other organisms. The growing demand for animal protein stimulates an expansion of production systems, increasing the stocking density. Outdoor poultry breeding systems (organic or not) that enable lower population density and higher animal welfare does not exclude these animals the presence of environmental pathogens. The control of gastro-intestinal helminthosis in non-organic intensive and extensive systems is accomplished by administering anthelmintics with high cost and results unsatisfactory due to the misuse of drugs with consequent selection parasite strains resistant to chemical bases. This problem stimulate research into alternative control measures. Nematophagous fungi are used by its enzymatic action in controlled conditions and how environmental biocontrolers of larvae of gastrointestinal nematodes of livestock. This study evaluated the capacity of conidia/chlamydospores of nematophagous fungi as *Duddingtonia flagrans* (AC001 and CG722) and *Monacrosporium thaumasium* (NF34A) for cross the gastrointestinal tract of domestic chickens (*Gallus gallus domesticus*), and yours germination after traffic and predatory activity "*in vitro*" on larvae of *Panagrellus* spp. Fungi conidia/chlamydospores was identified in feces of chickens at times of 6, 12 and 24 hours after administration and spores viability was found after observing the germination, mycelial growth, followed by production of traps, capture and death of *Panagrellus* spp larvae in feces. Fungi Nematophagous are alternative control measures, efficient and innovative technology for the biological control of helminth parasites of chickens.

Keywords: biological control, *Duddingtonia flagrans*, nematophagous fungi, *Monacrosporium thaumasium,* poultry

RESUMO

Parasitas são comuns em sistemas intensivos avícolas ou orgânicos, mais propício para o surgimento de parasitas gastrointestinais, favorecido pelo contato direto dos animais com o solo e outros organismos. O aumento da demanda por proteína animal estimula a expansão dos sistemas de produção com aumento da densidade animal. Sistemas de criação livres (orgânicos ou não) com baixa densidade e alto padrão tecnológico não excluem estes animais do contato com patógenos ambientais. O controle das helmintoses gastrointestinais em sistemas extensivos ou intensivos não orgânicos é

realizado pela administração de anti helmínticos com elevado custo e resultados insatisfatórios devido ao mau uso dos produtos com consequente seleção de cepas parasitárias resistentes às bases químicas. Este problema estimula a pesquisa de medidas alternativas de controle. Fungos nematófagos são utilizados por sua atividade enzimática em condições controladas e como biocontroladores de larvas de namatóides gastrointestinais em animais. Este estudo avaliou a capacidade de conídios/clamidósporos dos fungos nematófagos *Duddingtonia flagrans* (AC001 e CG722) e *Monacrosporium thaumasium* (NF34A) passar pelo trato gastrointestinal de galinhas domésticas (*Gallus gallus domesticus*), a germinação após passagem e atividade predatória "*in vitro*" sobre larvas de *Panagrellus* spp. Conídios/clamidósporos fúngicos foram identificados nas fezes das aves nos tempos 6, 12 e 24 horas após administração e a viabilidade dos esporos foi contatada após germinação, crescimento micelial, seguido pela produção de armadilhas, captura e destruição de larvas de *Panagrellus* spp. nas fezes. Fungos nematófagos são medidas alternativas de controle, uma eficiente tecnologia e inovadora para o controle biológico de helmintos parasitas de aves.

Palavras-chave: avicultura, controle biológico, *Duddingtonia flagrans*, fungos nematófagos, *Monacrosporium thaumasium*

INTRODUCTION

The Brazilian production of chicken meat has increased considerably and the country is now the world's third largest producer and leader in exports (RODRIGUES et al., 2014). The growing demand for animal protein stimulates a rapid expansion of production systems and increasing the stocking density, favoring the multiplication and spread of pathogens. Outdoor poultry breeding systems that enable lower population density and higher animal welfare does not exclude these animals the presence of environmental pathogens (SOBRAL et al., 2010).

Parasites are common in intensive or organics systems of chickens, which is more conducive to the emergence of gastrointestinal parasites, favored by direct contact with soil and other organisms (RUFF, 1999; SHEIKH et al., 2015, VIEIRA et al., 2015). Helminths are responsible for economic losses due to decreased food intake, reduced growth and posture, leading to nutritional deficiencies that makes the birds susceptible to other infectious diseases and can lead to death (CARDOZO & YAMAMURA, 2004; UHUO et al., 2013; HASSAN et al., 2015; THAPA et al., 2015).

The control of gastro-intestinal helminthosis in non-organic intensive and extensive systems is accomplished by administering anthelmintics with high cost and results unsatisfactory due to the misuse of drugs with consequent selection parasite strains resistant (THOMAZ-SOCCOL et al., 2004; AHMAD et al., 2013; SILVA et al., 2015). Contaminating the environment and serving as a source of contagion to other birds, generate residues in animal products (HERD, 1996; SHARMA et al., 2015).

The search for products contamination-free chemical implies the no use of medication, either for prophylaxis or treatment of diseases. Nematophagous fungi are used by its enzymatic action in controlled conditions and as environmental biocontrolers of larvae of gastrointestinal nematodes of livestock (THAMSBORG et al., 1999; BRAGA & ARAÚJO, 2014; HOSTE et al., 2015).

The basic principle for future biochemical experiments is to check the bioactivity of fungal structures after passage through the gastrointestinal tract (GIT) of the animal. This study evaluated the capacity of conidia/chlamydospores of *D. flagrans*

(Dudd.) R.C. Cooke (strains AC001 and CG722) and *M. thaumasium* (Drechsler) de Hoog & Oorschot (strain NF34A) through the gastrointestinal tract of domestic chickens (*Gallus gallus domesticus*), yours germination after the passage and predatory activity "*in vitro*" on larvae of *Panagrellus* spp.

MATERIAL AND METHODS

The experiment was conducted in the dependencies of the Laboratory of Parasitology of Department of Veterinary, Federal University of Viçosa (DVT/UFV) in Viçosa - MG.

Two isolates of *D. flagrans* (AC001 and CG722) and one of *M. thaumasium* (NF34A) belonging to mycology collection of the Parasitology Laboratory of DVT/UFV, kept in culture tubes AA2% (Water Ágar 2%) at 4ºC in the dark were transferred in Petri dishes of 9.0cm diameter containing CMA 2% (Corn Meal Ágar 2%) then incubated in an BOD (Biochemical Oxygen Demand) chamber at 25°C for 28 days. Ten mL of destilled water were added to the plate with the fungus grow, with the aid of a brush the fungal spores were removed, collected in a beaker and the volume completed to 10 mL. After homogenization, five aliquots of 10 uL of this solution were evaluated in a Neubauer chamber for quantification and identification of conidia/ chlamydospores according to the key proposed by Liu & Zhang (1994) and Van Oorschot (1985). The average of these counts was extrapolated the average-number of reproductive structures in solution, after quantified were diluted in distilled water to a concentration of 6.4×10^4 spores per mL and stored at 4°C.

Panagrellus spp maintained in culture in petri dishes containing oat flakes in Parasitology Laboratory DVT/UFV were washed 5 times with distilled water and centrifuge 500G for 5 minutes. After they were aliquoted 500 larvae. Eight chickens (*G. Gallus domesticus*), with approximately 6 months of age and average weight of 2.750 kg were kept in cages lined with plastic floor and divided into treatment groups, receiving commercial feed autoclaved and water "*ad libitum*".

The study was conducted in assay A and B. The assay A was evaluated the ability of fungal structures (conidia/chlamydospores) passing through the TGI of chickens and germinated in Petri dishes containing AA2%. In the assay B was evaluated the feasibility and predatory activity of fungi after passage through the GIT of chickens. The ability of fungal structures passing through the chicken GIT was evaluated at different times over a period of 3 days. The eight chickens were divided in pairs to make four experimental groups (G1, G2, G3 and G4). Every seven days was administered individually to each bird as a single dose orally 1 mL of aqueous solution containing 6.4×10^4 spores of isolates CG722, AC001, NF34A and distilled water without the presence of fungal structures (control group), in Latin Square design (SAMPAIO, 2007). In times of 6, 12, 24, 48 and 72 hours after administration fungi, fecal samples were collected with the aid of plastic bags on the floor of the cages, and then cleaning was carried. Fecal samples were collected at different times, homogenized by treatment group and about 2 grams spread on Petri dishes of 9.0 cm diameter containing AA2%. Approximately 500 larvae *Panagrellus* spp were added to plates and these put in a BOD chamber at 25°C for 12 days.

Every day the plates were examined under optical microscopy to visualize the presence of fungal structures and/or mycelial growth as described by Liu & Zhang (1994) and Van Oorschot (1985). At the end of this period the larvae not preyed was recovered by the Baermann technique.

The average number of larvae recovered, after logaritimic transformation (log x+1), was subjected to analysis of variance and compared by Tukey test at 5% significance with BioEstat software 5.3 (AYRES et al., 2003).

RESULTS AND DISCUSSION

The fungal structures (conidia/chlamydospores) the isolates CG722, AC001 and NF34A was identified in feces at intervals of 6, 12 and 24 hours after administration, confirming the ability to resist the passage through the GIT of *G. gallus domesticus*. The resistance of spores (conidia and chlamydospores) of fungal isolates (AC001, CG722 and NF34A) to pass through GIT of *G. gallus domesticus* and its recovery in the feces, suggest possible use of these organisms as biological controllers of the helminths, since Faedo & Waller (1996) and Herd (1996) describe the growing demand for healthy foods free of drug residues.

The fungal growth in the test "*in vitro*" (Assay B) with consequent production mycelial and traps, followed by capture and predation of *Panagrellus* spp larvae confirms the viability of fungal structures after passage through GIT of chickens (Figure 1) and its predatory activity, corroborating with Braga et al. (2012) that showed the crude extract of *P. chlamydosporia* (isolated VC1 and VC4) reduced 64.1 and 56.5%, respectively, the number of eggs of *A. galli*, demonstrating that nematophagous fungi may be used as biological controllers, especially in agroecologic systems which seek to produce healthy food of high nutritional value and free of contaminants, preserving biodiversity in which it operates production system.
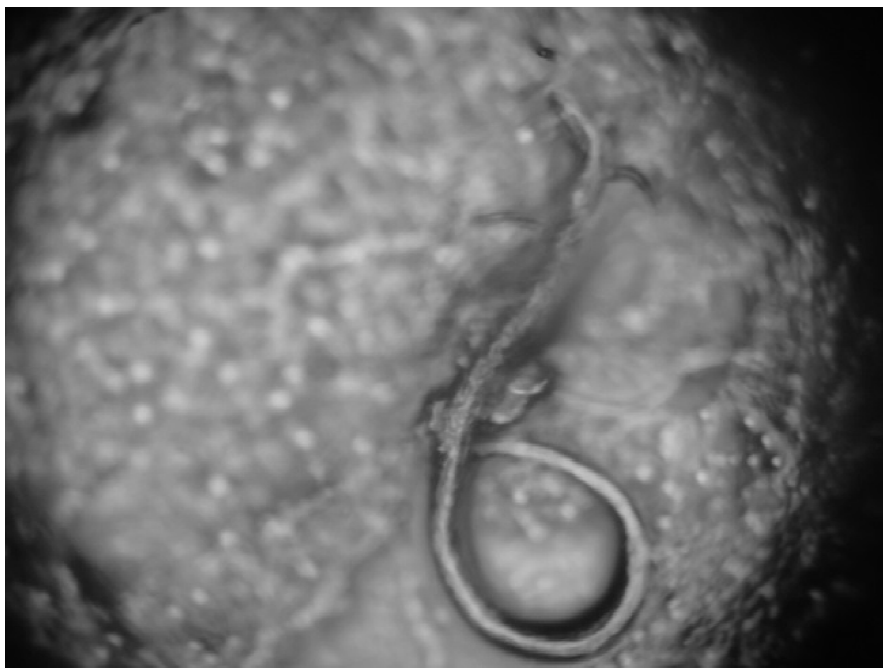
Figure 1. *D. flagrans*-traps (AC001) preying *Panagrellus* spp larvae in Petri dishes with AA2% - 40x magnification

The best percentage of reduction *Panagrellus* spp was the time 6 hours after administration fungal. At this time, the isolated AC001, CG722 and NF34A had, respectively, a reduction of 70.57 (p<0.05), 34.98 and 65.89% of the number of *Panagrellus* spp larvae (Figure 2 and Table 1) agreeing to Braga et al. (2013) also demonstrated in the test "*in vitro*" that *D. flagrans* (AC001 and CG722) and *A. cladodes* (CG719) were efficient in preying L₃ *Libyostrongylus douglassii* after seven days, reducing by 85.2% (AC001), 81.2% (CG722) and 89.2% (CG719) the average number of L₃ recovered. Genier et al. (2015) demonstrated the enormous applicability of biological control by *Pleurotus ostreatus* (PLO06) and their proteases on larvae of *Panagrellus* spp with reduction of 65.6, 77.4 and 95.2% after 24, 48 and 72 hours, respectively.

Although most predatory activity has been observed in time six hours after administration for all fungal isolates, only the isolated AC001 showed a statistically significant difference (p<0.05) compared to control. Hiura et al. (2015) showed that

D. flagrans (AC001) and *P. chlamydosporia* (VC4) were effective in reducing the infectivity of eggs and tissue migration of *Toxocara canis* for chickens (*G. gallus domesticus*) after previous contact with them, reducing 87.1% (AC001) and 84.5% (VC4) the number of eggs recovered from liver, muscle, lung and intestine. Thapa et al. (2015) demonstrate on water agar that *P. chlamydosporia* Biotype 10 had reduced the viability of *A. gall* and *T. canis* eggs by 64–86% and 26–67%, may be utilised as a biocontrol agent to reduce contamination of the environment, corroborating the idea of our work.

Once spores were visualized in the stool until the time 24 hours after its administration was not observed reduction in the number of larvae *Panagrellus* spp from the time 48 or 72 hours in the reduction test of larvae (Assay B) by any of the fungal isolates (p<0.05) (Table 1). This observation provides biosecurity standard, reducing environmental flood by nematophagous fungi.

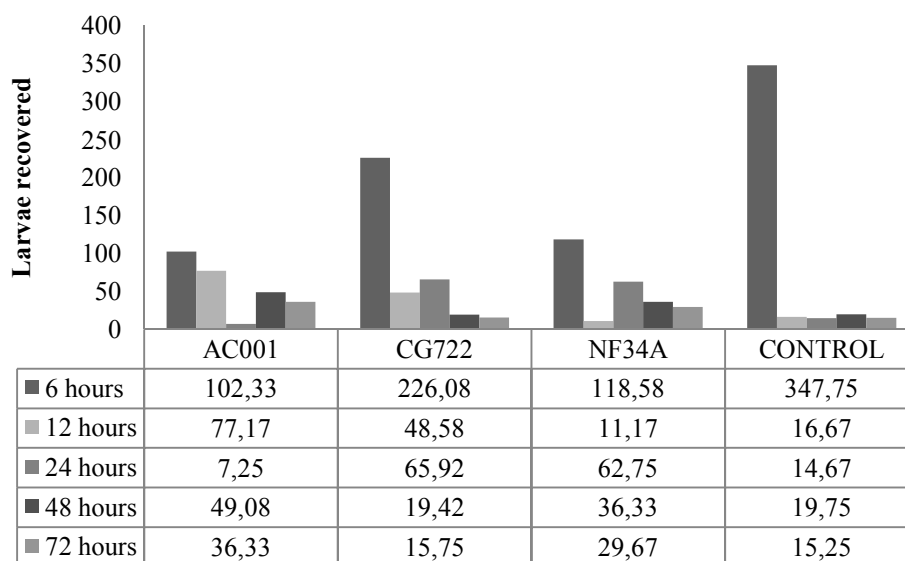|	AC001	CG722	NF34A	CONTROL
6 hours	102,33	226,08	118,58	347,75
12 hours	77,17	48,58	11,17	16,67
24 hours	7,25	65,92	62,75	14,67
48 hours	49,08	19,42	36,33	19,75
72 hours	36,33	15,75	29,67	15,25

Figure 2. Destruction of *Panagrellus* spp larvae by *D. flagrans* (AC001 and CG722) and *M. thaumasium* (NF34A) after passage through the gastrointestinal tract of *G. gallus domesticus*

Table 1. Number average, standard deviation and % reduction of *Panagrellus* spp larvae recovered to Baermann technique 12 days after interaction with *D. flagrans* (AC001 and CG722) and *M. thaumasium* (NF34A) in Petri dishes

Time	Parameters	AC001	CG722	NF34A	CONTROL
6 hours	Number average Standard deviation	102,33 (±111,02)[a]	226,08 (±297,85)[ab]	118,58 (±91,21)[ab]	347,75 (±290,82)[b]
	% reduction	-70.57	-34.98	-65.89	0
12 hours	Number average Standard deviation	77,17 (±96,35)[a]	48,58 (±47,60)[ab]	11,17 (±17,07)[bb]	16,67 (±24,89)[ab]
	% reduction	+362.9	+191.49	-33.99	0
24 hours	Number average Standard deviation	7,25 (±7,26)[a]	65,92 (±81,00)[a]	62,75 (±38,25)[ab]	14,67 (±23,06)[bb]
	% reduction	-50.56	+349.43	+327.83	0
48 hours	Number average Standard deviation	49,08 (±38,31)[a]	19,42 (±20,01)[a]	36,33 (±35,70)[a]	19,75 (±22,11)[a]
	% reduction	+148.5	-1.6	+83.94	0
72 hours	Number average Standard deviation	36,33 (±46,36)[a]	15,75 (±15,08)[a]	29,67 (±24,01)[a]	15,25 (±13,84)[a]
	% reduction	+138.22	+3.27	+94.55	0

Diferent small letters in lines mean statistically significant difference (p<0.05) - Tukey test.

Use of nematophagous fungi as biological controllers of helminth parasites of chickens is an efficient technology, innovative and low cost with possibility of use on all production systems, especially in conventional organic systems.

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