TRAPPING CAPABILITY OF *Arthrobotrys* sp AND *Monacrosporium thaumasium* ON CYATHOSTOME LARVAE

Capacidade predatória de *Arthrobotrys* sp e *Monacrosporium thaumasium* sobre larvas de ciatostomíneos

RODRIGUES[1] M.L.A., CASTRO[2] A.A., OLIVEIRA[2] C.R.R., ANJOS[2] D.H.S., BITTENCOURT[1] V.R.E.P. & ARAÚJO[3] J.V.

(1) Prof Adj. Deptº. Parasitologia Animal-UFRRJ, lurdesar@ufrrj.br- Univ.Fed.Rural do Rio de Janeiro Br 465 Km 7 Seropédica – RJ – 23890-000; (2) Curso de Pós-Graduação Ciências Veterinárias; (3) Prof Adj. Deptº.Veterinária UFV-MG

SUMMARY: Experiments were performed to determine the predacious capacity of *Arthrobotrys robusta* (I-35), *A. robusta* (I-31), *A. musiformis* (I-40) and *Monacrosporium thaumasium* on cyathostome larvae of equines. After seven days of interaction, no induction of trapping with free-living nematode larvae (*Panagrellus redivivus*), *M. thaumasium* captured 99% of the larvae. *A. robusta* (I-35) 69.4%; *A. robusta* (I-31) 79.3% and *A. musiformis* 77%. The species of fungi observed demonstrated predatory capacity on cyathostome larvae, and *M. thaumasium* was the most efficient.

EY WORDS*: Monacrosporium thaumasium, Arthrobotrys robusta, A. musiformis,* Cyathostominae, equine, predatory capacity.

INTRODUCTION

Nematophagous fungi are able to capture and destroy free-living nematodes. They occur in many different taxonomic groups (BARRON, 1977) and are found in soil, faeces of domestics animals and organic matter in decomposition (GRAY, 1983). These characteristics attracted interest in explorer fungus ability in controlling nematode larvae. Studies appraising the action of the nematophagous fungi on free-living stages of nematodes of bovines, ewes and equines have obtaining excellent results (PANDEY, 1973; GRONVOLD *et al*. 1987, 1993 a,b; CHARLES *et al.* 1995; LARSEN *et al*. 1992, 1996; SANTOS *et al.* 2001).

The equines are hosts of a great number of helminths, that complete their evolutionary cycle in the large intestine, and among them the strongylides are considered the most important and frequent (OGBOURNE & DUNCAN, 1985). The cyathostome are responsible for many intestinal diseases (HERD, 1990), and it is rare to find a equine without small strongylid (KOHER Jr., 1998).

The parasite control program in equines must try to prevent the infection, as the best result come from the preventive treatments. The strongest worm infection occur during the dry period of the year (winter), through the ingestion of the larvae in the pasture during the raining period. At summer the larvae on the ground develop, making the pasture contaminated and dangerous to the animals, specially to the young (KOHER Jr., 1998; SOULSBY, 1987).

Cyathostome presented resistance to benzimidazols and an efficient anthelmintic substance to control this parasite has not been found. Biological control is an important alternative to solve the anthelmintic resistance (BIRD & HERD, 1995; CHARLES *et al.* 1995).

This study aims to evaluate and compare "*in vitro*" predatory capacity of four fungi isolates, *Arthrobotrys robusta* (I-31), (I-35), *A. musiformis* (I-40) and *Monacrosporium thaumasium* (NF 34[a]) on infective larvae of cyathostome.

MATERIAL AND METHODS

This study was performed at Helmintology Laboratory at the Station for Parasitological Research W. O. Neitz–

This investigation was supported by grants from CNPq and CAPES.

Departament of Animal Parasitology at Universidade Federal Rural do Rio de Janeiro. Crossbred equines (*Equus caballus*) cyathostome naturally infected were maintained on pasture *Paspalum notatum*.

The four strains of nematophagous fungi used were *Arthrobotrys robusta* (I-31), *Arthrobotrys robusta* (I-35), *A. musiformis* (I-40) and *Monacrosporium thaumasium* (NF 34[a]). These strains were obtained from Veterinary Department at Federal University of Viçosa (MG – Brazil).

Feces from donors were collected directly from the rectum of the animal. Three random samples were taken from the fecal mixture and FEC was determined by the mean of the of three counts. Fecal cultures (total number = 30) were performed as follow: about 40g of faeces were mixed in a 200 ml plastic cup, which had holes in the bottom for aeration. The cup was wrapped with a petri dish, with a piece of paper between it.

After seven days, the cup received water until it's full, then was wrapped with the dish and finally turned over. The dish received 10 ml of water, to migration of third stage larvae. After two hours of baermannization, the water in the dish was carefully suctioned off to leave a volume less than 10 ml. The cup containing the culture material was discarded (ROBERTS & O´SULLIVAN, 1950).The water and residue containing L_3 were observed at microscopic quantified and identified (BEVILAQUA *et al.* 1993).

Recovered larvae were washed through centrifugation with sterilized and distilled water during ten minutes and 1500 rpm. Eleven washes like that were done, but at fifth, distilled water was changed for antibiotic solution (0.05% of chloramphenicol, 0.05% of streptomycin sulfate, 0.05% of amphotericin B) (ARAÚJO, 1996). After washes, the number of larvae per ml was quantified and estimated by extrapolation to obtain a suspension of 1000 larvae / ml. The first 100 L_3 encountered in each sample were identified to subfamily.

The medium to inoculate, growth and development of the fungi was composed of 20g of water agar solved in 1000ml of distilated water and 0.05g of chloramphenicol, it was mixed and sterilized during 20 minutes at 120ºC and 1 atm.

Under Laminar Flux Chamber, fifty sterilized dishes (5 cm ø) received 10 ml of culture medium per dish and were separated in five groups. Each group was composed with ten dishes. Treatments groups: I (*A. robusta* – I-31); II (*A. robusta* – I-35), III (*A. musiformis* – I-40), IV (*M. thaumasium* – NF34a) and V (no fungi) control group. All dishes were maintained at climactic chamber at 26º C and ± 85% of relative humidity (Araújo *et al*. 1996).

After seven days, 1 ml of suspension larvae was inoculated in all dishes, and the groups returned to the climactic chamber at same conditions of temperature and humidity above, during others seven days. After this period, the dishes were observed and evaluated at optic microscopic and the number of captured larvaes was counted.

The results were analyzed with T Student's Test ($P< 0,05$) Zar (1998).

RESULTS AND DISCUSSION

Results of apprehension are given in Table I, and at control group there was no larvae apreended. *M. thaumasium* was the best in apprehension capability (99.2%), with significant difference ($P\leq 0.05$) observed among other isolates, *A. robusta* (I-31), (I-35) and *A musiformis* (I-40) did not differ between them. These results are according to Pandey (1973), that observed 100% of apprehension of *Trichostrongylus axei* and *Ostertagia ostertagi* L_3 through *M. thaumasium*.

NANSEN *et al.* (1986) observed quickly imobilization and death of free-living nematodes *Panagrellus redivivus* and *Rhabditis wohlgemuthi* and of L_1 and L_2 of *Cooperia oncophora* by the fungi *A. oligospora,* but L_3 of *C. oncophora* was alive after 20 hours. At the present study, it was observed that seven days after interaction larvae of cyathostome were apprehend, 79.3% by *A. robusta* (I-31); 69.4% by *A. robusta* (I-35) and 77% by *A. musiformis* (I-40) (Table I); and a few number of larvae continued alive and free. Some authors have induced trapping of fungi with free-living nematodes (Nansen *et al.* 1988); however, at the present study, there was no use of free-living nematodes to induce trapping only cyathostome larvae being used to induce the trapping. This fact may justify

TABLE I: Groups, total number, number of captured larvae and apprehension percentual.

Groups	Total ± σ	Number of larvae Captured ± σ	Variance(σ^2) (%)*	Apprehension
I (*A.robusta* I-35)	585 ± 186.6	406 ± 173.0	0.0231	69.4[b]
II *(M. thaumasium)*	515 ± 215.0	511 ± 215.0	0.001	99.2[a]
III (*A robusta* I-31)	353 ± 211.0	280 ±234.0	0.0467	79.3[b]
IV*(A. musiformis* I-40*)*	235 ± 89.0	181 ± 93.2	0.0155	77.0[b]

σ Standard deviation

*Results followed by the same letters don't differ at of 5% of probability.

the variation on the reduction percentage (69 and 79% by *Arthrobotrys* spp and 99% by *M. tahumasium*) when compared with NANSEN (1988).

Strains of *A. musiformis* from South of Minas Gerais State, Brazil, apprehended 100% of *P. redivivus* larvae between the 4th and 6th day of interaction (NAVES & CAMPOS, 1991); that same fungi reduced 100% of *Haemonchus placei* after 20 days of interaction (ARAÚJO *et al.* 1994). At the present study, the same isolates apprehended 77% of cyathostome larvae after seven days of interaction, a similar result to Mendonza de GIVES *et al.* (1992) to *Haemonchus contortus.* Predatory capability may be different between isolates of same species or genera (ARAÚJO *et al.* 1992; 1993); however, between strains (I-31) and (I-35) of *A. robusta* there was no significant difference (Table I).

Monacrosporium thaumasium, in a preliminary study, after three days interaction with cyathostome L_3, presented 27.34% of apprehension (RODRIGUES *et al*. 1999). However, this study, presented 99.2% after seven days of interaction, demonstrating that apprehension increased with time.

All species of this study demonstrated predatory capability to cyathotome larvae, but *M. thaumasium* (NF34a) was the most efficient, similar to what was observed by MOTA *et al.* (1999) on *H. contortus* larvae.

According to WALLER (1997), studies *in vitro* are important, but at the present there is a few information of nematophagous fungi in environment conditions, so it is necessary to realize more studies with predacious fungi in field on faeces pastures, with passage through gastrintestinal tract of animals. That same author commented that the first step beginning to select fungi to biological control of helminths is to obtain it from soil isolates from different regions of the country were the study is being developed**.**

This experiment has shown the nematode trapping fungus *M. thaumasium* has potential as a biological control agent for Cyathostominae, a pathogenic gastrointestinal nematodes in horses.

SUMÁRIO

Experimentos foram realizados para determinar a capacidade predatória de *Arthrobotrys robusta* (I-35), *A. robusta* (I-31), *A musiformis* (I-40) e *Monacrosporium thaumasium* sobre larvas de ciatostomíneos de eqüinos. Após sete dias de interação, sem pré-indução de armadilhas com larvas de nematóides de vida livre ((*Panagrellus redivivus)*, *M taumasium* capturou 99% das larvas; *A. robusta* (I-35), 69,4%; *A. robusta* (I-31), 79,3% e *A musiformis 77%.* Todas as espécies de fungos avaliados demonstrara, capacidade predatória sobre as larvas de ciatostomíneos, porém *M taumasium* foi a mais eficiente.

PALAVRAS CHAVE*: Monacrosporium thaumasium, Arthrobotrys robusta, A. musiformis,* ciatostomíneos, eqüinos, capacidade predatória.

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