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Fungi extract in the inhibition of the egg hatching and larval development of the *Haemonchus contortus*

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***In vitro* efficacy of aqueous and ethanolic extracts of *Paecilomyces lilacinus* and *Trichoderma longibrachiatum* was assessed in the egg hatching inhibition (EHI) and larval development inhibition (LDI) of *Haemonchus contortus.* For the EHI assessment, after 48 h of incubation with different concentrations of aqueous and ethanolic extracts, it was quantified blastomeres, embryonated eggs, and larvae of first stage. For the LDI assessment, a quantitative coculture adapted method was used. After seven days of feces incubation with different concentrations of aqueous extract of fungi, the infective larvae were collected and quantified, obtaining the number of developed larvae per feces gram. The aqueous extract of *P. lilacinus* at 1.96 mg mL^{-1} and the ethanolic extract of *T. longibrachiatum* at 1.90 mg mL^{-1} completely inhibited the egg hatching. In the LDI, the aqueous extract of *T. longibrachiatum* at 1.90 mg mL^{-1} showed efficacy of 92.88%. These fungi extracts showed potential to inhibition of the *H. contortus* cycle.**

Key words: *Paecilomyces lilacinus*, *Trichoderma longibrachiatum*, alternative control, sheep breeding, gastrointestinal nematodes.

INTRODUCTION

Sheep are bred worldwide on family farms and for commercial agriculture, contributing to hides, meat, wool, and milk. *Haemonchus contortus* is the most common parasite in sheep reared in tropical areas. Animals with

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hemonchosis may show anemia and submandibula swelling, with high mortality in young lambs and peripartal ewes. Both sexes at all age levels may be infected, decreasing weight gain and feed conversion efficiency; reduced meat, wool, and milk production; and reproductive disorders (Sczesny-Moraes et al., 2010; Taylor et al., 2010).

The frequent treatment with synthetic antihelminthics is the main method of control. However, it has promoted rapid selection of resistant helminthes populations (Duarte et al., 2012; Adiele et al., 2013; Soro et al., 2013). Resistance has been observed to the main classes of antihelminthics in different continents, impeding the animal breeding (Taylor et al., 2009). Thus, the search for alternatives is being widely done in order to decrease animal infection, the contamination of animal products, the environment and the human beings by chemical antihelminthics residues.

Researches using fungi as a biological control have shown that this alternative consists of a promising control method of gastrointestinal nematodes (Araujo et al., 2004). Nevertheless, the development of fungal formulations economically practicable and easily applicable is a challenge in the implementation of the fungal species in programs of biological control (Campos et al., 2007).

Paecilomyces lilacinus can infect eggs and adult females of Meloidogyne spp. nematode and causes death of embryos in 5 to 7 days. This fungus has given excellent results under varying conditions (Mukhtar et al., 2013). Similarly, *Trichoderma longibrachiatum* has been found to be an effective biocontrol agent for the management nemateded of root-knot and other plant nematodes. The mechanisms of *T. longibrachiatum* against nematodes are essentially unknown (Zhang et al., 2014).

Little is known about the effect of these fungi extracts in the ruminant gastrointestinal nematodes cycle. In this paper, it was assessed the efficacy of the extracts of the fungi *P. lilacenus* and *T. longibrachiatum* in the inhibition of egg hatching and of the larval development of *H. contortus*.

MATERIALS AND METHODS

Study area

The research was conducted in a rural area in city of Montes Claros in the north of Minas Gerais state, Brazil (W 43°50'33.56"; S 16°41'10.05"). The climate of the region is tropical wet with dry summer (As) according to the Köppen classification (Alvares et al., 2013), is marked by a long dry season from May to October and a rainy period in January and February.

Molecular identification

Isolated fungi were obtained from the rectum of the sheep matrix of the breed Santa Inês raised in tropical field (Freitas et al., 2012). Extraction of total DNA of microorganisms was performed according to Rosa et al. (2009). The primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) were used for amplification of the rDNA region of aerobic mycelia fungi, as described by White et al. (1990). The primers used for yeasts were NL1 (5'-GCA TAT CAA AAG GAA GAG TAA GCC-3') and NL4 (5'-GGT AAG CTT GCA TGT CCG G-3'). The Polymerase Chain Reaction (PCR) was performed and amplicons produced were purified and assayed in NanoDrop ND 1000 (NanoDrop Technologies) as reported in Abrão et al. (2014). The sequencing reactions were performed in DYEnamicTM (Amersham Biosciences, USA) associated with automated sequencing system MegaBACETM 1000 (Lachance et al., 1999). The sequences of the rDNA fragments were analyzed according to Altschul et al. (1997), in the BLASTN program (Basic Local Alignment Search Tool - 2215 BLAST version 2.0) from the National Center for Biotechnology. Isolates with similarity ≥99% to a previously deposited sequence were considered to belong to the same species. After identification, the cultures were preserved as Castellani (1967).

Sample collection and aqueous and ethanolic extracts

The fungi were previously cultivated in plates containing solid Agar Sabouraud (Sabouraud Dextrose Agar, Prodimol Biotechnology, MG, Brazil) during seven days at 28°C. It was used as inoculum five discs of about 5 mm of diameter of the fungal cultures transferred to Erlenmeyers containing liquid Sabouraud, with no yeast extract. It was incubated in *termoshaker* at 30°C and 150 rpm during seven days. After this period, the cultivations were filtered twice in Whatman n° 1 paper and in Millipore membrane with 0.22 µm of porosity, using vacuum pump. The liquid obtained of each culture, called aqueous extract, was used immediately, and the fungal mass was frozen at -4°C.

To obtain the ethanolic extracts, an adapted method from Nery et al. (2010) was used. 100 mL of ethyl alcohol P.A. (99.5º GL) was added to each 50 g of fungal mass. The mixture was homogenized and transferred to a beaker tighten with plastic wrap and incubated in bain-marie at 40°C for 90 min. Later, it was filtrated through two Whatman nº 1 papers. The extracts obtained were placed in Petri plates for dehydration in an incubator with forced air circulation at 40 ± 5°C for three days and stored in freezer at -4°C.

The dry matter (DM) of the extract subsamples was determined at 105°C according to Association of Official Analytical Chemists (AOAC, 1990), to the tested concentrations calculus (Cunniff, 1995).

Egg hatching inhibition

Flotation, sedimentation, and filtration in saturated NaCl solution were conducted to obtain nematode eggs from feces (Coles et al., 1992) of two Santa Inês lambs infected with *H. contortus* and with an average fecal egg count of >1000 g^{-1}, determined using the modified McMaster technique (Gordon and Whitlock, 1939). The procedures were done in accordance with the ethical principles of the animal experimentation, approved in the 42/2008 protocol of the Ethics Committee on the Animal Experimental of the Federal University of Minas Gerais.

The treatments were conducted with five replicates: Positive control with levamisole phosphate (0.3 mg mL^{-1}); negative control with sterile purified water; and experimental treatments comprising four concentrations of each extracts (Tables 1 and 2).

Experimental mixtures comprised 100 µL of fecal suspension with an average of 80 fresh eggs and 100 µL of the extracts. The samples were homogenized and incubated in a BOD incubator at 28°C for 48 h. Subsequently, 15 µL of Lugol's solution was added to each tube, which were then stored at 4°C for counting of unembryonated and embryonated eggs, and stage one larvae (L1)

Table 1. Effects of aqueous extract and ethanolic extract of *P. lilacenus* on hatchability of *Haemonchus contortus.*

Treatment (mg mL-^{1})	Unembryonated eggs mean	Embryonated eggs mean	L1 mean	Efficacy* (%)
Aqueous extract				
1.96	142.00	8.50	0.00^{a}	100.00
3.92	130.00	4.25	0.00^{a}	100.00
5.89	126.25	4.25	0.00^{a}	100.00
7.85	116.75	6.50	0.00^{a}	100.00
Levamisole phosphate (0.3 mg mL^{-1})	97.50	39.50	0.00^{a}	100.00
Sterile distilled water	12.75	18.00	86.00^{b}	-
Variation coefficient (%)			47.22	
Ethanolic extract				
8.40	18.50	65.25	21.00^{b}	79.05
16.80	3.00	139.25	0.75^{a}	99.25
25.19	2.50	153.75	0.75^{a}	99.25
33.59	4.25	76.25	0.50^{a}	99.55
Levamisole phosphate (0.3 mg mL-^{1})	19.50	45.00	0.00	100.00
Sterile distilled water	0.25	0.50	100.25^{c}	-
Variation coefficient (%)			27.27	

Different letters in columns indicates significant differences by Tukey's test ($P < 0.05$). *% EHI = 100 × (1 - L1 of treatment / L1 of control).

Table 2. Effects of aqueous extract and ethanolic extract of *T. longibrachiatum* on hatchability of *H. contortus.*

Treatment (mg mL^{-1})	Unembryonated eggs mean	Embryonated eggs mean	L1 mean	Efficacy *(%)
Aqueous extract				
0.59	128.50	121.25	34.5^{c}	89.63
1.17	9.50	16.75	93.25^{b}	71.97
1.76	6.75	10.25	98.00^{b}	70.54
2.35	3.00	9.25	46.00^{b}	86.17
Levamisole phosphate (0.3 mg mL^{-1})	262.00	4.50	0.00^{c}	100.00
Sterile distilled water	2.00	0.75	332.75^{a}	-
Variation coefficient (%)			45.70	
Ethanolic extract				
1.90	23.25	12.75	0.00^{a}	100.00
3.81	9.50	3.50	0.00^{a}	100.00
5.71	16.25	11.00	0.25^{a}	99.49
7.62	30.5	21.25	2.25^{a}	95.38
Levamisole phosphate (0.3 mg mL^{-1})l	50.75	10.25	0.00^{a}	100.00
Sterile distilled water	0.75	0.00	48.75^{b}	-
Variation coefficient (%)			14.15	

Different letters in columns indicates significant differences by Duncan test ($P < 0.05$). *% EHI = 100 × (1 - L1 of treatment / L1 of control).

(Coles et al., 1992).

The egg count and L1 was transformed into relative values for the initial number of eggs for replicate. The means were compared using Tukey's test ($P < 0.05$). Probit regression was employed to determine the concentrations sufficient to inhibit 50% (LC50) and 90% (LC90) of egg hatching with SAEG software (SAEG, 2007). The percent of egg hatching inhibition (EHI) and larval development was calculated using the formula modified after Coles et al. (1992):

*% EHI = 100 × (1 - L1 of treatment / L1 of control).

Inhibition of larval development

To evaluate the effectiveness of aqueous extract of *P. lilacinus* and *T. longibrachiatum* on larval development inhibition (LDI), the quantitative coproculture method (Borges, 2003; Nery et al., 2010;

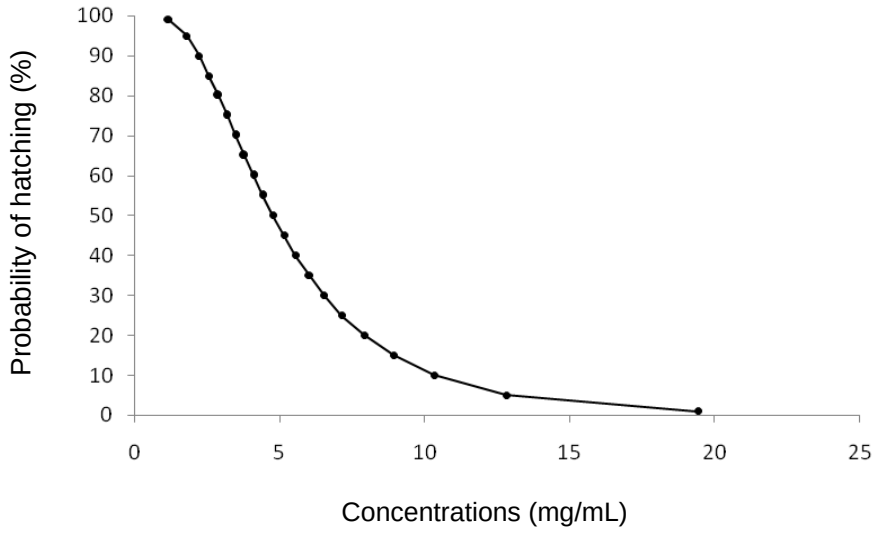

Figure 1. Probability of hatching inhibition of *H. contortus* at different concentrations of the ethanolic extract of *P. lilacenus.*

Nogueira et al., 2012) was employed using feces of lambs with *H. contortus* mono-infection.

Treatments were conducted, each with five replicates, including positive controls with 2 mL of 16 µg g^{-1} ivermectin solution (LA Ranger, Vallée, Minas Gerais, Brazil) added to 2 g of feces, and negative control of 2 mL of sterile purified water (negative control). The experimental treatments consisted of aqueous extract of *P. lilacinus* and *T. longibrachiatum* standardized at 0.28 and 1.13 mg g^{-1} and 0.20 and 0.79 mg dried weight (dw) g^{-1}, respectively.

The cultures were incubated in a BOD incubator at 28°C for seven days and assessed for presence of infective larvae (L3). The following formula, adapted from Borges (2003), was used to determine the percent reduction of larvae (L3) per gram of feces (LPGF):

% LDI = 100 × (1 - LPGF of the treated group/LPGF of the untreated group).

The data was log transformed, log (x+1), and submitted to variance analysis. The means were compared by the Duncan's test (P < 0.05). The LC50 and LC90 were determined by probit analysis with SAEG software (SAEG, 2007).

RESULTS AND DISCUSSION

Sequence analysis of rDNA revealed the presence of *P. lilacinus* and *T. longibrachiatum* with 99.9% of similarity. These species have been used largely in the control of plants nematodes and they are producers of lytic enzymes, which are able to destroy parasitical structures (Ferreira et al., 2008; Shuwu et al., 2015; Huang et al., 2016).

For *P. lilacinus*, it verified efficacy of 100% in the EHI in all concentrations of aqueous extract (1.96 to 7.85 mg mL^{-1}) and efficacy superior to 99% in the concentrations ≥ 16.8 mg mL^{-1} of the ethanolic extract (Table 1). The LC_{50} and LC_{90} obtained in the egg hatching inhibition, using ethanolic extract, were 4.77 and 10.31 mg mL^{-1}, respectively (Figure 1). This way, it is suggested that these fungus metabolites can have effect in the inhibition of the embryonic development and in the egg hatching inhibition of *H. contortus* (Zhang et al., 2014).

It was not possible to estimate the LC_{50} and LC_{90} for EHI experiments with a aqueous extract of the *P. lilacinus* because the low tested concentration showed 100% of efficacy. For the aqueous extract and ethanol from *T. longibrachiatum* it was observed a dose-dependent response.

For the ethanolic extract of *T. longibrachiatum,* all evaluated concentrations presented significant reduction in the number of L1 when related to the distilled water control with efficacy superior to 95% (Table 2). The aqueous extract at 0.59 mg $mℓ^{-1}$ presented significant reduction in the average of L1 larvae hatched, and all concentrations reduced the total number of present eggs and larvae after 48 h of incubation, when compared to the distilled water control (Table 2). Thus, it is suggested that enzymes or the fungus metabolites could have caused degradation in these structures, destroying them completely and impeding the microscopic examination.

In the inhibition of larval development, the concentration of 0.79 mg g^{-1} of the aqueous extract of *T. longibrachiatum* promoted a reduction ($p<0.05$) in the average of the infective larvae with efficacy ≥ 92% (Table 3), showing that, even in fecal material, the metabolites presented lethal effect for the eggs and the nematode larvae. Due to the low productivity in dry matter to the aqueous extract of *P. lilacinus* used in the quantitative coproculture, the concentrations used were too low, not obtaining relevant efficacy (Table 3).

To all experiments using the aqueous and ethanolic extracts of *P. lilacinus* and *T. longibrachiatum,* it was

Table 3. Effectiveness of the aqueous extract of *P. lilacenus* and *T. longibrachiatum* on the larval development of *H. contortus* in quantitative fecal cultures.

Treatment (mg g^{-1})	LPGF*	Efficacy* (%)
P. lilacenus		
0.28	108.00^{b}	22.46
0.56	93.00^{b}	32.83
0.84	94.00^{b}	32.04
1.13	92.00^{b}	34.05
Ivermectin (16 µg g^{-1})	0.00^{a}	100.00
Sterile distilled water	139.00^{b}	-
Variation coefficient (%)	5.47	
T. longibrachiatum		
0.20	346.00^{c}	2.40
0.39	358.00^{c}	0.00
0.59	332.00^{c}	6.43
0.79	26.00^{b}	92.78
Ivermectin (16 µg g^{-1})	0.00^{a}	100.00
Sterile distilled water	354.00^{c}	-
Variation coefficient (%)	3.66	

Different letters in column indicates significant differences by Tukey test ($P < 0.05$). * % LDI = 100 × (1 - LPGF of the treated group/LPGF of the untreated group). * LPGF, number of larvae (L3) g^{-1} of feces.

verified eggs and larvae L1 with structural deformity in the barks and cuticles. The distilled water controls performed embryonic and normal larval development to the nematode. Some fungi species can destroy nematodes with toxin action (Nordbring-Hertz, 1988) and others can produce metabolites with ovicidal effects (Waller and Faedo, 1993). Nematophagous fungi can penetrate the cuticle structure of the nematode larvae because they have proteases, chitinases and collagenases involved directly in the nematode infection process (Gryndler et al., 2003). According to Braga et al. (2011), the fungus *Pochonia clamydosporia* produces a protease that destroys the eggs of fitonematodes and of *Ancylostoma* spp.

Fungi are known to have huge metabolic diversity providing a wide range of commercial compounds, including many antibiotics used in medicine. Furthermore, metabolites produced by fungi may have potential nematicides, being targets of research in biotechnology (Smedsgaard and Nielsen, 2005). A new peptide extracted of *Omphalotus olearius* fungus has demonstrated similar action to commercial nematicide Ivermectin (Mayer et al., 1997). Review by Li et al. (2007) showed 179 compounds isolated from fungi that have demonstrated activity as nematicide groups of alkaloids, peptides terpenoids, macrolide compounds, oxygen heterocycle and benzo compounds, aliphatic compounds, simple aromatic compounds.

These metabolites and enzymes with ovicidal and larvicidal action were already identified in fungi from the genus *Aspergillus* and *Trichoderma.* The species *Aspergillus niger* produces citric acid, chymosin, lipase, protease and α-amylase (Bennett, 1998; Jiang and An, 2000; Meyer, 2008). According to De Marco et al. (2000), *Trichoderma* spp. are producers of proteases, hydrolases, chitinases, exoglucanases and endoglucanases of the type ß- glucanases (ß-1,3 and ß-1,6), and cellulases (ß-1,4- D-glucosidases), substances that perform important enzymatic activity in the biocontrol.

According to Ferreira et al. (2008), the species *P. lilacinus* is a parasite of eggs and of female adults of fitonematodes and present great relevance to the biological control. Devrajan and Seenivasan (2002) observed that *P. lilacinus* presented toxic effect to the adult fitonematodes from the genus *Melodoidyne.* This fungal species also performed efficacy *in vitro* on *Taenia saginata* eggs in conditions at the end of ten days (Braga, 2008) and on *Toxocara canis* eggs after seven days of treatment (Araújo et al. 2004).

On the commercialized bioproducts in relation to the control of pathogenic agents causers of diseases in plants, there are three bioproducts based on *A. flavus* for the fungi control, 11 based on *Paecilomyce s*sp. for the nematodes control and 55 based on *T. longibrachiatum* for the fungi and bacteria control (Sosa-Gomez, 2002). As regards the commercialized bioproducts in relation to the control of pathogenic agents that causes diseases in

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Conclusion

The aqueous extracts of *P. lilacinus* and ethanolic of *T. longibrachiatum* at low concentrations inhibited completely egg hatching. The aqueous extract of *T. longibrachiatum* ≥ 0.79 mg mL^{-1} showed efficacy ≥ 92% to LDI of *H. contortus*. The isolated ones from these fungi were obtained naturally from the digestive tract of sheep and in future studies the probiotic effect of these species must be evaluated on the *in vivo* inhibition of the cycle of gastrointestinal nematodes.

Conflict of Interests

The authors of this manuscript have no financial or personal relationship with individuals or organizations that could influence or bias the content of the paper. All procedures were performed in accordance with the principles of animal experimentation approved in the 275/2013 protocol of the Ethics Committee on the use of animals (CEUA) of the Federal University of Minas Gerais, Brazil.

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REFERENCES

Abrão FO, Duarte ER, Rosa CA, Freitas CES, Vieira EA, Huhges AFS (2014). Characterization of fungi from ruminal fluid of beef cattle with different ages and raised in tropical lignified pastures. Curr. Microbiol. 69(2):1-13.

Adiele RC, Fakae B, Isuzu IU (2013). Anthelmintic 413 activity of *Securidaca longepedunculata* (Family: Polygalaceae) root extract in mice, *in vitro* and *in vivo*. Asian Pac. J. Trop. Med. 6:841-846.

Altschul SF, Madden TL, Schaffer AA, Zheng Zhang JZ, Miller W, Lipman DJ (1997). Gapped BLAST and PSI−BLAST: a new generation of protein database search programs. Nucleic Acid Res. 25:3389-3402.

Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM, Sparovek G (2013). Köppen's climate classification map for Brazil. Meteorologische Zeitschrift 22:711-728.

Araújo JV, Assis RCL, Alves PH, Campos AK, Gandra JR (2004). Controle biológico de tricostrongilídeos (Nematoda: Trichostrongyloidea) gastrintestinais de bovinos pelo fungo *Monacrosporium sinense*. Arq. Bras. Med. Vet. Zoot. 56(4):467-471.

Bennett JW (1998). Mycotechnology: the role of fungi in biotechnology. J. Biotechnol. 66:101-107.

Borges CCL (2003). Atividade in vitro de anti-helmínticos sobre larvas infectantes de Nematodeos gastrintestinais de caprinos, utilizando a técnica de coprocultura quantitativa (Ueno, 1995). Parasitol. Latinoam. 58(3/4):142-147.

Braga FR, Araújo JV, Araújo JM, Carvalho RO, Silva AR (2008). Efeito do fungo Paecilomyces lilacinus sobre ovos de *Taenia saginata*. Rev. Soc. Bras. Med. Trop. 41(6):686-688.

Braga FR, Araujo JM, Silva ARE, Araújo JV, Carvalho RO, Soares EF, Queiroz JH, Gênier HJA (2011). Ação ovicida do extrato bruto enzimático do fungo *Pochonia chlamydosporia* sobre ovos de *Ancylostoma* sp. Rev. Soc. Bras. Med. Trop. 44(1):116-118.

Campos AK, Araújo JV, Assis RCL, Gandra JR, Guimarães MP (2007). Viabilidade de formulação peletizada do fungo nematófago *Monacrosporium sinense*, no controle biológico de nematóides parasitos gastrintestinais de bezerros. Arq. Bras. Med. Vet. Zootec. 59(1):14-20.

Castellani AA (1967). Maintenance and Cultivation of the common pathogenic fungi of man in sterile distilled water. Further researches. J. Trop. Med. Hyg. 70(1):181-184.

Coles GC, Bauer C, Borgsteede FH, Geerts S, Klei TR, Taylor MA, Waller PJ (1992). World Association for the Advancement of Veterinary Parasitology (WAAVP) – methods for detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44:35-44.

Cunniff P (Eds.) (1995). Official methods of analysis of AOAC International**.** Washington: AOAC, 2000 p.

De Marco JL, Lima LHC, Souza MV, Feliz CRA (2000).*Trichoderma harzianum* chitinase destroys the cell wall of the phytopathogen *Crinipellis perniciosa*, the causal agent of witches' broom disease of cocoa. World J. Microbiol. Biotechnol. 16:383-386.

Devrajan K, Seenivasan N (2002). Biochemical changes in banana roots due to *Meloidogyne incognita* infected with *Paecilomyces lilacinus*. Curr. Nematol. 13:1-5.

Duarte ER, Silva RB, Vasconcelos VO, Nogueira FA, Oliveira JF (2012). Diagnóstico do controle e perfil de sensibilidade de nematódeos de ovinos ao albendazol e ao levamisol no norte de Minas Gerais. Pesq. Vet. Bras. 32(2):147-152.

Ferreira PA, Ferraz S, Lopes EA, Freitas LG (2008). Parasitismo de ovos de *Meloidogyne exigua* por fungos nematófagos e estudo da compatibilidade entre os isolados fúngicos. Rev. Trop. Cienc. Agrarias Biol. 2 (13):15-21.

Freitas CES, Abrão FO, Silva KL, Almeida PNM, Duarte ER (2012). Fungos aeróbios no intestino grosso de borregos e de ovelhas criados em pastagens tropicais. Arq. Bras. Med. Vet. Zootec. 64:225-227.

Gordon HM, Whitlock HV (1939). A new technique for counting nematode eggs in sheep faeces. J. Counc. Sci. Ind. Res. 12:50-52.

Gryndler M, Jansa J, Hrselová H, Chvátalová, L, Vosátka M (2003). Chitin stimulates development and sporulation of arbuscular mycorrhizal fungi. Appl. Soil Ecol. 22(1):283-287.

Huang J, Lu C, Qian X, Huang Y, Zheng Z, Shen Y (2016). Effect of salinity on the growth, biological activity and secondary metabolites of some marine fungi. Acta Oceanol. Sin. 30(3):118-123.

Jiang ZD, An Z (2000). Bioactive fungal natural products through classic and biocombinatorial approaches. Stud. Nat. Prod. Chem. 22:245-272.

Lachance MA, Bowles JM, Starmer WT (1999). *Kodamacea kakaduensi* and *Candida tolerans*, two new ascomycetous yeast species from Australian Hibiscus flowers. Can. J. Microbiol. 45:172-177.

Li G, Zhang K, Xu J, Dong J, Liu Y (2007). Nematicidal Substances from Fungi. Recent Pat. Biotechnol. 1(3):213-233.

Mayer A, Anke H, Sterner O (1997). Omphalotin, a new cycle peptide with potent nematicidal activity from *Omphalotus olearius*. I. Fermentation and biological activity. Nat. Prod. Lett. 10:25-32.

Meyer V (2008). Genetic engineering of filamentous fungi–Progress, obstacles and future trends. Biotechnol. Adv. 26(1):177-185.

Mukhtar T, Arshad Hussain M, Zameer Kayani M (2013). Biocontrol potential of *Pasteuria penetrans*, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Trichoderma harzianum* against *Meloidogyne incognita* in okra. Phytopathol. Mediterr. 52(1):66-76.

Nery PS, Nogueira FA, Martins ER, Duarte ER (2010). Effects of *Anacardium humile* leaf extracts on the development of gastrointestinal nematode larvae of sheep. Vet. Parasitol. 171:361-364.

Nogueira FA, Fonseca LD, Silva RB, Ferreira AVP, Nery PS, Geraseev LC, Duarte ER (2012). *In vitro* and *in vivo* efficacy of aqueous extract of *Caryocar brasiliense* Camb. to control gastrointestinal nematodes in sheep. Parasitol. Res. 111:325-330.

Nordbring-Hertz B (1988). Nematophagous fungi: strategies for nematode exploitation and for survival. Microbiol. Sci. 5:108-116.

Rosa LH, Vaz ABM, Caligiorne RB, Campolina S, Rosa CA (2009). A Endophytic fungi associated with the Antarctic grass *Deschampsia antarctica* Desv (Poaceae). Polar Biol. 32:161-167.

SAEG (2007). Sistema para Análises Estatísticas, Versão 9.1: Fundação Arthur Bernardes - UFV – Viçosa.

Sczesny-Moraes EA, Bianchin I, Silva KF, Catto JB, Honer MR, Paiva F (2010). Anthelmintic resistance of gastrointestinal nematodes in sheep, Mato Grosso do Sul, Brazil. Pesq. Vet. Bras. 30:229-236.

Shuwu Z, Gan Y, Bingliang XB (2015). Potencial de biocontrole de uma espécie nativa de *Trichoderma longibrachiatum* contra *Meloidogyne incognita.* Ecologia do Solo Aplicada 94:21-29.

Smedsgaard J, Nielsen J (2005). Metabolite profiling of fungi and yeast: from phenotype to metabolome by MS and informatics. J. Exp. Bot. 56:273-286.

Soro D, Koné WM, Bonfoh B, Dro B, Toily KB, Kamanzi K (2013). *In vivo* anthelmintic activity of *Anogeissus leiocarpus* Guill & Perr (Combretaceae) against nematodes in naturally infected sheep. Parasitol. Res. 112:2681-2688.

Sosa-Gomez DR (Eds.) (2002). Fungos entomopatogênicos: catálogo de isolados. Londrina: Embrapa Soja 1(1):1-32.

Taylor MA, Coop RL, Wall RL (Eds.) (2010). Parasitologia Veterinária. Guanabara Koogan, Rio de Janeiro. 742 p.

Taylor MA, Learmount J, Lunn E, Morgan C, Craig BH (2009). Multiple resistance to anthelmintics in sheep nematodes and comparison of methods used for their detection. Small Rumin. Res. 86:67-70.

Waller PJ, Faedo M (1993). The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: screening studies. Vet. Parasitol. 49:285-297.

White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In. Innis M.A., Gelfand D.H., Sninsky J.J., White T.J. (Eds), PCR Protocols: A Guide to Methods and Applications. San Diego, Academic Press. pp. 315-322.

Zhang S, Gan Y, Xu B, Xue Y (2014). The parasitic and lethal effects of *Trichoderma longibrachiatum* against *Heterodera avenae*. Biol. Control 72:1-8.