MARIA LARISSA BITENCOURT VIDAL

AÇÃO de *Duddingtonia flagrans* COMPARADA A ASSOCIAÇÃO DE *Duddingtonia flagrans* E *Pochonia chlamydosporia* NO CONTROLE BIOLÓGICO DE NEMATOIDES DE BOVINOS E CRESCIMENTO DE FUNGOS NEMATÓFAGOS NA PRESENÇA DE ANTI-HELMÍNTICOS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Medicina Veterinária, para obtenção do título de *Doctor Scientiae*.

Orientador: Jackson Victor de Araújo

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RESUMO

VIDAL, Maria Larissa Bitencourt, D.Sc., Universidade Federal de Viçosa, setembro de 2024. **Ação de *Duddingtonia flagrans* comparada a associação de *Duddingtonia flagrans* E *Pochonia chlamydosporia* no controle biológico de nematoides de bovinos e crescimento de fungos nematófagos na presença de anti-helmínticos.** Orientador: Jackson Victor de Araújo.

A criação de bovinos apresenta influência pelos nematoides gastrointestinais, sendo a prática fundamental para a economia brasileira. Os objetivos do estudo foram avaliar a ação dos fungos *Duddingtonia flagrans* e *Pochonia chlamydosporia* na redução dos helmintos gastroinstestinais de bovinos criados extensivamente em pastagens, avaliar ação e atividade nematicida dos fungos *D. flagrans* (isolado AC001) e *P. chlamydosporia* (isolado VC4), por meio da contagens de ovos de helmintos parasitos por grama de fezes (OPG) e as reduções do número de larvas infectantes (L3) recuperadas das coproculturas das fezes e da pastagem, além de avaliar o crescimento dos fungos *D. flagrans* (isolado AC001), *P. chlamydosporia* (isolado VC4) e *Arthrobotrys.musiformis* (isolado I144) sob efeito *in vitro* de abamectina, albendazol e levamisol. A avaliação da atividade nematicida dos fungos foi realizada com dezoito bovinos machos Holstein x Zebu, com idades entre 12 e 15 meses, foram divididos em três grupos de seis animais: T1, T2 e controle. O grupo T1 recebeu uma formulação oral contendo *D. flagrans*, e o grupo T2 recebeu uma formulação contendo *D. flagrans* e *P. chlamydosporia* e o controle que não recebeu tratamento. Os animais foram mantidos em cercados naturalmente infestados com larvas de nematoides (L3). Durante nove meses, a carga parasitária foi monitorada por meio de amostragens fecais e coleta de pasto para determinar a infestação de L3 no ambiente. Devido à baixa precipitação durante o experimento, a recuperação de L3 das pastagens foi baixa. No entanto, a contagem de ovos por grama de fezes (OPG) foi significativamente menor nos grupos T1 e T2 em comparação ao grupo controle em abril, maio e julho. Em março, os valores foram menores apenas no grupo T1 em relação ao controle. A administração dos fungos na dosagem de 6 g/100 kg de peso vivo, contendo 10^6 clamidósporos de cada fungo por grama, reduziu tanto o OPG quanto a infestação de L3 nas pastagens, indicando a eficácia dos fungos no controle dos nematoides gastrintestinais. Na influência de anti-helmínticos comerciais sobre o crescimento de fungos nematófagos. Foram testados os anti-helmínticos cloridrato de levamisole 7,5% (Ripercol®), solução de abamectina 1% (Calbos®) e solução de

sulfóxido de albendazole 15% (Agebendazole®). As formulações foram diluídas em quatro níveis de concentração: 1, 10, 100 e 1000 ppm para albendazol e abamectina, e 2;23; 22,3; 223 e 2230 ppm para levamisole. Os tratamentos foram acompanhados por controles: negativo (meio de cultura PDA 2%), positivo (meio de cultura com estirpe fúngica) e de água. O crescimento dos fungos *A. musiformis*, *D. flagrans* e *P. chlamydosporia* foi analisado em esquema fatorial com três repetições. Os resultados mostraram que *A. musiformis* e *P. chlamydosporia* sofreram menor influência dos anti-helmínticos em comparação a *D. flagrans*, que apresentou maior inibição de crescimento. O albendazol, nas concentrações de 1 e 10 ppm, teve maior impacto no crescimento dos fungos. Esses resultados indicam a necessidade de compreensão detalhada da viabilidade das associações entre anti-helmínticos e fungos nematófagos para o estabelecimento de protocolos eficazes de controle integrado de nematoides gastrointestinais.

Palavras-chave: Bovinocultura. Controle integrado. Fungos helmintófagos. Redução de helmintos.

ABSTRACT

VIDAL, Maria Larissa Bitencourt, D.Sc., Universidade Federal de Viçosa, August, 2024. **Action *Duddingtonia flagrans* compared to the association of *Duddingtonia flagrans* and *Pochonia chlamydosporia* in the biological control of cattle nematodes and growth of nematophagus fungus in the presence of antihelminthics.** Adviser: Jackson Victor de Araújo.

Cattle farming is influenced by gastrointestinal nematodes, and this practice is fundamental to the Brazilian economy. The objectives of the study were to evaluate the action of the fungi *Duddingtonia flagrans* and *Pochonia chlamydosporia* in reducing the gastrointestinal helminths of cattle raised extensively on pasture, to evaluate the action and nematicidal activity of the fungi *D. flagrans* (isolate AC001) and *P. chlamydosporia* (isolate VC4), by means of parasitic helminth egg counts. By means of parasitic helminth egg counts per gram of feces (EPG) in faeces and reductions in the number of infective larvae (L3) recovered from coprocultures of faeces and pasture, as well as assessing the growth of the fungi *D. flagrans* and *P. chlamydosporia* and *Arthrobotrys musiformis* (isolate I144) under the in vitro effects of abamectin, albendazole and levamisole. The evaluation of the nematicidal activity of the fungi was carried out on eighteen Holstein x Zebu male cattle, aged between 12 and 15 months, which were divided into three groups of six animals: T1, T2 and control. The T1 group received an oral formulation containing *D. flagrans*, the T2 group received a formulation containing *D. flagrans* and *P. chlamydosporia* and the control received no treatment. The animals were kept in pens naturally infested with nematode larvae (L3). For nine months, the parasite load was monitored through fecal sampling and pasture collection to determine the infestation of L3 in the environment. Due to the low rainfall during the experiment, the recovery of L3 from the pastures was low. However, the egg count per gram of feces (EPG) was significantly lower in groups T1 and T2 compared to the control group in April, May and July. In March, the values were lower only in group T1 compared to the control. The administration of the fungi at a dosage of 6 g/100 kg live weight, containing 10^6 chlamydospores of each fungus per gram, reduced both OPG and L3 infestation in the pastures, indicating the effectiveness of the fungi in controlling gastrointestinal nematodes. The influence of commercial anthelmintics on the growth of nematophagous fungi. The anthelmintics tested were levamisole hydrochloride 7.5% (Ripercol®), abamectin solution 1% (Calbos®) and albendazole sulphoxide solution 15% (Agebendazole®). The formulations were diluted

at four concentration levels: 1, 10, 100 and 1000 ppm for albendazole and abamectin, and 2.23, 22.3, 223 and 2230 ppm for levamisole. The treatments were accompanied by controls: negative (2% PDA culture medium), positive (culture medium with the fungal strain) and water. The growth of the fungi *A. musiformis*, *D. flagrans* and *P. chlamydosporia* was analysed in a factorial design with three replicates. The results showed that *A. musiformis* and *P. chlamydosporia* suffered less influence from anthelmintics compared to *D. flagrans*, which showed greater growth inhibition. Albendazole, at concentrations of 1 and 10 ppm, had the greatest impact on fungal growth. These results indicate the need for a detailed understanding of the viability of associations between anthelmintics and nematophagous fungi in order to establish effective integrated control protocols for gastrointestinal nematodes.

Keywords: Bovine farming. Helminthophagous fungi. Helminth reduction. Integrated control.

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LISTA DE SIGLAS E ABREVIATURAS

AA	Ágar água.
ABZ	Albendazole.
AC001	*Duddingtonia flagrans.*
BDA	Batata dextrose ágar.
BOD	Biological Oxygen Demand.
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.
CEUA	Comitê de Ética no Uso de Animais.
CLSI	Clinical and Laboratory Standards Institute.
CMA	Corn Meal Ágar.
GIN	Gastroinstestinal nematodes.
GIT	Gastroinstestinal tract.
EF	Experimental formulation.
EPG	Egg counts per gram of feces.
IBGE	Instituto Brasileiro de Geografia e Estatística.
I144	*Arthrobotrys musiformis*
L3	Larvas Infectantes.
PDA	Potato dextose ágar.
SINDAN	Sindicato Nacional da Indústria de Produtos para Saúde Animal.
VC04	*Pochonia chlamydosporia.*
WAAVP	World Association for the Advancement of Veterinary Parasitology.

LISTA DE SÍMBOLOS

®	Marca Registrada
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1. INTRODUÇÃO GERAL

A criação de bovinos desempenha um papel crucial na economia brasileira, sendo o Brasil, o país detentor do maior rebanho comercial de gado do mundo (IBGE 2023) e terceiro maior produtor mundial de leite (BRASIL 2023). A bovinocultura garante um grande potencial para a produção de leite e carne, com destaque a pecuária leiteira produzindo no ano de 2022, 34.609.218 mil litros de leite, com arrecadação de 80.043.813 mil reais, sendo Minas Gerais o estado com maior produção (IBGE 2023).

A criação dos bovinos, predominantemente é realizada de forma extensiva nas pastagens (Beretta et al. 2002; Dias-Filho, 2016), favorecendo a ocorrência constante de infecções por nematoides gastrintestinais (NGI), que prejudicam o desempenho dos animais e provocam perdas econômicas significativas, estimadas em cerca de US $ 7,11 bilhões anuais (Grisi et al. 2014).

Os NGIs mais comuns nos rebanhos brasileiros pertencem aos gêneros *Haemonchus* e *Cooperia* (Oliveira et al. 2018; de Oliveira et al. 2022; Vieira et al. 2020a). O ciclo de vida destes parasitos envolve uma fase de vida livre no ambiente e uma fase parasitária no animal (Taylor et al. 2016). No ambiente, os ovos eliminados nas fezes evoluem para larvas infectantes (L3), que migram para a pastagem e são ingeridas pelos animais, iniciando a fase parasitária até se tornarem vermes adultos (Girão et al. 1999).

No cenário contemporâneo da criação de ruminantes, há uma ênfase crescente no aumento dos níveis de produção e na garantia do bem-estar animal (Fonseca et al. 2023). Nesse contexto, o controle de NGIs é crucial (Grisi et al. 2014).

O controle das helmintíases é realizado predominantemente com o uso de anti-helmínticos (Vidal et al. 2019; Kaplan, 2020), sendo as lactonas macrocíclicas, como a abamectina, os benzimidazóis, como albendazol e os imidotiazóis, como o levamisole, são os grupamentos químicos amplamente utilizado e difundidos no mercado nacional (Delgado et al. 2009).

No entanto, a administração desses medicamentos não elimina as fases de vida livre dos parasitos, que permanecem viáveis no bolo fecal e nas pastagens (Wang et al. 2020; Voinot et al. 2020). Alternativas para integrar ao controle químico, com ação direta nas fases de vida livre, são os bioprodutos, destacando-se o uso de fungos

nematófagos como método seguro e viável (Braga et al. 2020; Luns et al. 2018; Vilela et al. 2018).

Os fungos nematófagos, como *Duddingtonia flagrans*, *Pochonia chlamydosporia* e *Arthrobotrys musiformis*, têm demonstrado eficácia no controle de larvas e ovos de helmintos, respectivamente (Braga e Araújo 2014; Mendonza-de-Gives et al. 2018; Vieira et al. 2020a; Araújo 2023). Inúmeros resultados têm sido obtidos com várias espécies de fungos nematófagos, em especial *Duddingtonia flagrans* para o controle de larvas de helmintos e *Pochonia chlamydosporia* com ação ovicida para o controle de ovos de helmintos (Braga e Araújo 2014; Mendonza-de-Gives et al. 2018; Oliveira et al. 2018; Vieira et al. 2020a; Araújo et al. 2021; Oliveira et al. 2022; Araújo 2023).

Duddingtonia flagrans destaca-se pela capacidade de formar clamidósporos que sobrevivem à passagem pelo trato gastrointestinal dos animais, disseminando-se no ambiente através do bolo fecal e predando larvas L3 de nematoides com suas hifas adesivas (Gronvold et al. 1996; Buzatti et al. 2015; Braga et al. 2020; Vieira et al. 2020b; Fonseca et al. 2022; Li et al. 2022). Possui alta produção de esporos, que formam conídios e dão origem a hifas que tem o papel nematófago, que ao aprisionar os nematoides apresentam ação destrutiva, por produzir alta quantidade de clamidósporos, que coloniza e prende com facilidade e são altamente resistentes apresentando alta taxa de sobrevivência ambiental (Fonseca et al. 2022; Calazans et al. 2023).

Pochonia chlamydosporia atua principalmente na destruição de ovos de helmintos através da penetração mecânica e ação enzimática, colonizando e digerindo o conteúdo dos ovos (Lopes et al. 2007; Podestá et al. 2009; Araújo et al. 2021). Este fungo não forma armadilhas como *D. flagrans*, mas desenvolve apressórios que permitem a colonização e penetração na superfície dos ovos de helmintos (Lopez-Llorca et al. 2002; Araújo et al. 2021; Fonseca et al. 2023). É considerado facultativo parasitando ovos de nematoides, possui hifas septadas que constitui o micélio e produz esporos (Gams e Zaire 2001). As hifas possuem a capacidade de penetração por orifícios existentes na estrutura dos ovos (Araújo et al. 2004), há também relatos de *P. chlamydosporia* parasitando helmintos adultos (fêmeas) (Lopes et al. 2007).

Arthrobotrys musiformis é outro fungo nematófago que forma redes de hifas que capturam e digerem as larvas de nematoides. Sua ação predatória complementa

a dos outros fungos, oferecendo uma abordagem sinérgica no controle biológico dos helmintos (Vieira et al. 2019; Ayupe et al. 2016). É capaz de produzir armadilhas tridimensionais que imobilizam e promovem a adesão e penetração das larvas de helmintos (Oliveira et al. 2018). Estudos desmonstraram sua atividade em larvas de helmintos, isolado ou em associação a outros fungos, com alta capacidade predatória (Vieira et al. 2020b).

A utilização combinada de anti-helmínticos e fungos nematófagos em um programa de controle integrado pode ser uma estratégia vantajosa. O uso conjunto de métodos químicos e biológicos maximiza a eficácia no controle de NGI, reduzindo a população de parasitos no ambiente e prolongando a ação dos compostos químicos (Ortiz Pérez et al. 2017; Bilotto et al. 2018; Oliveira et al. 2018; Voinot et al. 2020; Araújo et al. 2021). No entanto, é crucial compreender a interação entre os anti-helmínticos e os fungos nematófagos, já que produtos sintéticos podem inibir o desenvolvimento e a esporulação desses fungos, comprometendo sua eficácia (Alizadeh et al. 1560; Neves et al. 2001).

Estudos têm indicado que os produtos sintéticos podem inibir o desenvolvimento dos fungos nematófagos (Alizadeh et al. 1560; Neves et al. 2001), o que pode comprometer a eficácia do controle biológico. Os anti-helmínticos podem eliminar resíduos nas fezes dos animais e isso tem grande relevância, uma vez que o bolo fecal é o principal local de ação dos fungos (Navrátilová et al. 2023). Além disso, os efeitos adversos das lactonas macrocíclicas em fungos que colonizam o estrume dos animais foram documentados (Junco et al. 2020). A integração de métodos de controle químico e biológico pode ser vantajosa, mas requer um conhecimento detalhado sobre a compatibilidade entre fungos nematófagos específicos e anti-helmínticos.

2. JUSTIFICATIVA E OBJETIVOS

A criação de bovinos desempenha um papel crucial na economia brasileira, que é predominantemente realizada de forma extensiva nas pastagens favorecendo a ocorrência constante de infecções por nematoides gastrintestinais, que prejudicam o desempenho dos animais e provocam perdas econômicas significativas.

O controle das helmintíases é crucial para a produtividade e acompanhamento sanitário dos rebanhos. Tradicionalmente, é realizado predominantemente com o uso de anti-helmínticos, no entanto, a administração desses medicamentos não elimina as fases de vida livre dos parasitos, que permanecem viáveis no bolo fecal e particularmente nas pastagens, além de outros fatores do próprio medicamento com efeitos no animal e no ambiente. Alternativas para integrar ao controle químico, com ação direta nas fases de vida livre, são o uso de fungos nematófagos que têm demonstrado eficácia no controle de larvas e ovos de helmintos.

Os bioprodutos, especialmente os fungos predadores, tem apresentado grande relevância com características promissoras e seguras, estão sendo notadas ações em ovos e larvas de helmintos, fases que não são controladas por moléculas químicas, o que demonstra ação fúngica de *Duddingtonia flagrans*, sua capacidade de formar clamidósporos que sobrevivem ao trato gastrointestinal dos bovinos, disseminando-se no ambiente e predando larvas L3 de nematoides com suas hifas adesivas. *Pochonia chlamydosporia*, por outro lado, destrói ovos de helmintos por meio de penetração mecânica e ação enzimática. Já *Arthrobotrys musiformis* forma redes de hifas que capturam e digerem larvas de nematoides, complementando a ação dos outros fungos. A utilização combinada de anti-helmínticos e fungos nematófagos em um programa de controle integrado pode aumentar a eficácia no manejo de NGIs, reduzindo a população de parasitos na fase ambiental, prolongando a ação dos anti-helmínticos.

A integração de métodos de controle químico e biológico pode ser vantajosa, mas requer um conhecimento detalhado sobre a compatibilidade entre fungos nematófagos específicos e anti-helmínticos. A escassez de estudos recentes sobre essa compatibilidade e a necessidade de padronização de métodos motivam a realização de pesquisas para investigar o efeito dos fármacos no crescimento de fungos nematófagos utilizados no controle biológico de helmintos.

2.1 Objetivo geral

Avaliar a ação dos fungos *Duddingtonia flagrans* e *Pochonia chlamydosporia* na redução dos helmintos gastrointestinais de bovinos criados extensivamente em pastagens do sudeste do Brasil.

2.2 Objetivos específicos

- Avaliar a ação e atividade nematicida dos fungos *D. flagrans* (isolado AC001) e *P. chlamydosporia* (isolado VC4),
- Avaliar as contagens de ovos de helmintos parasitos por grama de fezes (OPG) sob a ação dos fungos *D. flagrans* (isolado AC001) e *P. chlamydosporia* (isolado VC4).
- Avaliar as reduções do número de larvas infectantes (L3) recuperadas das coproculturas das fezes e da pastagem sob ação dos fungos *D. flagrans* (isolado AC001) e *P. chlamydosporia* (isolado VC4),
- Avaliar o crescimento dos fungos *D. flagrans* (isolado AC001), *P. chlamydosporia* (isolado VC4) e *Arthrobotrys.musiformis* (isolado I144) sob efeito *in vitro* de abamectina, albendazol e levamisol.

3. REFERÊNCIAS

ALIZADEH, A.; AMIN, M.; KHEZRI, M.; SABERI RISEH,R. Compatibility of *Beauveria bassiana* (Bals.) Vuill. with several pesticides. Int J Agric Biol 8530:1–9, 1560.

ARAÚJO JV. Advances in the control of helminthiases in domestic animals. Patogens 12: (9), 1107, 2023.

ARAÚJO, J.V.; BRAGA, F.R.; MENDOZA-DE-GIVES, P.; PAZ-SILVA, A.; VILELA, V.L.R. Recent Advances in the Control of Helminths of Domestic Animals by Helminthophagous Fungi. Parasitologia, v. 1, p. 168–176, 2021.

AYUPE, T.H.; MONTEIRO, T.S.A.; BRAGA, F.R.; SOARES, F.E.F.; MELLO, I.N.K.; ARAUJO, J.M.; FREITAS, L.G.; ARAÚJO, J.V. Assessment of compatibility between the nematophagous fungi Arthrobotrys robusta and Duddingtonia flagrans under laboratory conditions. Revista Iberoamericana de Micologia, v. 33, p. 129-130, 2016.

BERETTA, V.; LOBATO, J.F.P.; NETTO, C.G.M. Produtividade e eficiência biológica de sistemas de produção de gado de corte de ciclo completo no Rio Grande do Sul. Revista Brasileira de Zootecnia, v. 31, p. 991-1001, 2002.

BRAGA, F.R.; ARAÚJO, J.V. Nematophagous fungi for biological control of gastrointestinal nematodes in domestic animals. Applied Microbiology Biotechnology, v. 98, p. 71–82, 2014.

BRAGA, F.R.; FERRAZ, C.M.; SILVA, E.M.; ARAÚJO, J.V. Efficiency of the Bioverm® (Duddingtonia flagrans) fungal formulation to control in vivo and in vitro of Haemonchus contortus and Strongyloides papillosus in sheep. 3 Biotechnology, v. 10, p. 62-68, 2020.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento (MAPA). MAPA DO LEITE – MINISTÉRIO DE AGRICULTURA E PESCA. 2023. Disponível em: https://www.gov.br/agricultura/pt-br/assuntos/producao-animal/mapa-do-leite. Acesso em: 20 de julho 2024

BUZATTI, A.; DE PAULA SANTOS, C.; FERNANDES, M.A.; YOSHITANI, U.Y.; SPRENGER, L.K.; DOS SANTOS, C.D.; MOLENTO, M.B. Duddingtonia flagrans in the control of gastrointestinal nematodes of horses. Experimental Parasitology, v. 159, p. 1-4, 2015.

CALAZANS, F.B. et al. Avaliação do fungo *Duddingtonia flagrans* e hipoclorito de sódio a 5% sobre a eclodibilidade de ovos de ciatostomínaeos. Rev Med Vet 17:4, 225-229, 2023.

DELGADO, F.E.F.; LIMA, W.S.; CUNHA, A.P.; BELLO, A.C.P.P.; DOMINGUES, L.N.; WANDERLEY, R.P.B.; LEITE, P.V.B.; LEITE, R.C. Verminoses dos bovinos: percepção de pecuaristas em Minas Gerais, Brasil. Revista Brasileira de Parasitologia Veterinária, p. 18, v. 29-33, 2009.

DIAS-FILHO, M.B. Uso de Pastagens para a Produção de Bovinos de Corte no Brasil: Passado, Presente e Futuro. Documentos/Embrapa Amazônia Oriental, 42f, 2016. ISSN 1983-0513; 418.

FONSECA JS, ALTOÉ LSC, CARVALHO LM, SOARES FEF, BRAGAFR & ARAÚJO JV. Nematophagous fungus Pochonia chlamydosporia to control parasitic diseases in animals. App Microb Bio 107: 3859-3868, 2023.

FONSECA JS, FERREIRA VM, FREITAS SG, VIEIRA IS & ARAUJO JV. Efficacy of a Fungal Formulation with the Nematophagous Fungus Pochonia chlamydosporia in the Biological Control of Bovine Nematodiosis. Pathogens 11:6, 695; 2022. https://doi.org/10.3390/pathogens11060695

GAMS, W.; ZARE, R. A revision of Verticillium sect. Prostrata. III. Generic classification. Nova Hedwigia, v.72, p. 47-55, 2001.

GIRÃO, E.S.; LEAL, J.Á.; GIRÃO, R.N.; MEDEIROS, L.P. Verminose bovina. Teresina: Embrapa Meio-Norte/ Documentos 41, 30p, 1999.

GRISI, L.; LEITE, R.C.; MARTINS, J.R.S.; BARROS, A.T.M.; ANDREOTTI, R.; CANÇADO, P.H.D.; LEÓN, A.A.P.; PEREIRA, J.B.; VILLELA, H.S. Reassessment of the potential economic impact of cattle parasites in Brazil. Brazilian Journal Veterinary Parasitology, v. 23, p. 150-156, 2014.

GRONVOLD, J.; HENRIKSEN, S.A.; LARSEN, M.; NANSEN, P.; WOLSTRUP, J. Aspects of biological control with special reference to arthropods, protozoans and helminths of domesticated animals. Veterinary Parasitology, v. 64, p. 47-64, 1996.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA (IBGE) 2023 https://www.ibge.gov.br. Acesso em: 20 jul. 2024.

JUNCO, M.; IGLESIAS, L.E.; SAGUÉS, M.F. et al. Effect of macrocyclic lactones on nontarget coprophilic organisms: a review. Parasitol Res 120:773–783, 2021. https://doi.org/10.1007/s00436-021-07064-4

KAPLAN, R.M. Biology, epidemiology, diagnosis, and management of anthelmintic resistance in gastrointestinal nematodes of livestock. Veterinary Clinics North America Food and Animal Practice, v. 36, p. 17-30, 2020.

LI S, WANG D, GONG J & ZHANG Y. Individual and Combined Application of Nematophagous Fungi as Biological Control Agents against Gastrointestinal Nematodes in Domestic Animals. J Pathog 11: 172, 2022.

LOPES, E.A.; FERRAZ, S.; FERREIRA, P.A.; FREITAS, L.G.; DHINGRA, O.D.; GARDIANO, C.G.; CARVALHO, S.L. Potencial de isolados de fungos nematófagos no controle de Meloidogyne javanica. Nematologia Brasileira, v. 31, p. 78-84, 2007.

LOPEZ-LLORCA LV, OLIVARES-BERNABEU C, SALINAS J, JANSSON HB & KOLATTUKUDY PE. Pre-penetration events in fungal parasitism of nematode eggs. Mycol Res 106: 499–506, 2002.

LUNS FD, ASSIS RCL, SILVA LPC, FERRAZ CM, BRAGA FR & ARAÚJO JV. Coadministration of nematophagous fungi for biological control over nematodes in bovine in the South-eastern Brazil. Biomed Res Int 1-6, 2018.

MENDONZA-DE-GIVES P, LÓPEZ-ARELLANO MA, AGUILAR-MARCELINO L, OLAZARAN-JENKINS S, REYES-GUERRERO D, RAMÍREZ-VARGAS G & VEGA-MURLLO V. The nematophagous fungus *Duddingtonia flagrans* reduces the gastrointestinal parasitic nematode larvae population in faeces of orally treated calves maintained under tropical conditions - Dose/response assessment. Vet Parasitol 263, 66-72, 2018.

NAVRÁTILOVÁ, M.; VOKRAL, I.; KRÁTKY, J. MATAOUSKOVÁ, P. et al. Albendazole from ovine excrements in soil and plants under real agricultural conditions: Distribution, persistence, and efects. Chemosphere, 324, 138-343, 2023.

NEVES, P.M.O.J.; HIROSE, E.; TCHUJO, P.T.; MOINO, JRA.) Compatibility of entomopathogenic fungi with neonicotinoid insecticides. Neotrop Entomol 30:263–268, 2001. https://doi.org/10.1590/S1519-566X2001000200009

OLIVEIRA, I.C.; VIEIRA, I.S.; CARVALHO, L.M.; CAMPOS, A.K.; FREITAS, S.G.; ARAUJO, J.M.; BRAGA, F.R.; ARAÚJO, J.V. Reduction of bovine strongilides in naturally contaminated pastures in the southeast region of Brazil. Experimental Parasitology, v. 194, p. 9-15, 2018.

OLIVEIRA IC, VIEIRA IS, FREITAS SG, CAMPOS AK, & ARAÚJO JV. *Monascrosporium sinense* e *Pochonia chlamydosporia* para o controle biológico de larvas infectantes bovinas em pastagem de *Brachiaria brizantha*. Bio Control 171, 2022.

ORTIZ PÉREZ, D.O.; SÁNCHEZ MUÑOZ, B.; NAHED TORAL, J.; ORANTES ZEBADÚA, M.Á.; CRUZ LÓPEZ, J.L.; REYES GARCÍA, M.E.; MENDOZA DE GIVES, P. Using Duddingtonia flagrans in calves under an organic milk farm

production system in the Mexican tropics. Experimental Parasitology, v. 175, p. 74-78, 2017.

PODESTÁ, G.S.; DALLEMOLE-GIARETTA, R.; FREITAS, L.G.; LOPES, E.A.; FERRAZ, S. ZOOCA, R.J.F. Atividade nematófaga de Pochonia chlamydosporia em solo natural ou autoclavado sobre Meloidogyne javanica. Nematologia Brasileira, v. 33, p. 191-193, 2009.

TAYLOR, M.A.; COOP, R.L.; WALL, R.L. Veterinary Parasitology. Fourth Edition, WileyBlackwell, 1032p., 2016. ISBN: 978-0-470-67162-7

VIDAL MLB, VIANA MVG, ITO M, TRIVILIN LO & MARTINS IVF. Anti-helmínticos de importância veterinária no Brasil. In *Tópicos especiais em ciência animal VIII*; CAUFES: Alegre pp. 273–297, 2019.

VIEIRA, Í.S.; OLIVEIRA, I.C.; CAMPOS, A.K.; ARAÚJO, J.V. Association and predatory capacity of fungi Pochonia chlamydosporia and Arthrobotrys cladodes in the biological control of parasitic helminths of bovines. Parasitology, v. 146, p. 1347-1351, 2019.

VIEIRA, Í.S.; OLIVEIRA, I.C.; FREITAS, S.G.; CAMPOS, A.K.; ARAÚJO, J.V. Arthrobotrys cladodes and Pochonia clamydosporia in the biological controlo of nematodosis in extensive bovine production system. Parasitology, v. 147, p. 699-705, 2020a.

VIEIRA, Í.S.; OLIVEIRA, I.C.; CAMPOS, A.K.; ARAÚJO, J.V. In vitro biological control of bovine parasitic nematodes by Arthrobotrys cladodes, Duddingtonia flagrans and Pochonia chlamydosporia under different temperature conditions. Journal of Helminthology, v. 94, p. 1-6, 2020b.

VILELA VLR, FEITOSA TF, BRAGA FR, VIEIRA VD, LUCENA SC & ARAÚJO JV. Control of sheep gastrointestinal nematodes using the combination of *Duddingtonia flagrans* and levamisole hydrochloride 5%. Rev Bras Parasitol Vet 27: 23-31, 2018.

VOINOT, M.; CAZAPAL-MONTEIRO, C.; HERNÁNDEZ, J.A.; PALOMERO, A.M.; ARROYO, F.L.; SANCHÍS, J.; PEDREIRA, J.; SÁNCHEZ-ANDRADE, R.; PAZ-SILVA, A.; ARIAS, M.S. Integrating the control of helminths in dairy cattle:

Deworming, rotational grazing and nutritional pellets with parasiticide fungi. Veterinary Parasitology v. 278, 109038, 2020.

WANG, T.; AVRAMENKO, R.W.; REDMAN, E.M.; WIT, L.; GILLEARD, J.S.; COLWELL, D.D. High levels of third-stage larvae (L3) overwinter survival for multiple cattle gastrointestinal nematode species on western Canadian pastures as revealed by ITS2 rDNA metabarcoding. Parasite and Vectors, v. 13, p. 458, 2020.

4. CAPÍTULO 1

Efficacy of *Duddingtonia flagrans* and its combination with *Pochonia chlamydosporia* in controlling gastrointestinal nematodes in cattle

Efficacy of *Duddingtonia flagrans* and its combination with *Pochonia chlamydosporia* in controlling gastrointestinal nematodes in cattle

Abstract

This study evaluated the effects of oral administration of a formulation based on the fungus *Duddingtonia flagrans* (T1) and a formulation containing *D. flagrans* and *Pochonia chlamydosporia* (T2) for controlling gastrointestinal nematodes in naturally infected cattle. For nine months, eighteen male Holstein x Zebu cattle aged 12 to 15 months were divided into three groups of six animals each (T1, T2 and control), separated into paddocks naturally infested with nematode larvae (L3). The parasite load was monitored through fecal sampling and pasture collection to determine L3 infestation in the environment. Low L3 recovery from the pasture was observed due to low rainfall during the experiment (dry season). Egg counts per gram of feces (EPG) were lower in T1 and T2 than in the control group in April, May and July, while in March the values were lower only in T1 than in the control. Individual or combined administration of fungi at a dosage of 6 g/100 kg live weight of each product, containing 10^6 chlamydospores of each fungus per gram, reduced both EPG and L3 in pastures, thus indicating their effectiveness in controlling gastrointestinal nematodes in cattle.

Keywords: helminths; helmintophagous fungi; nematophagous fungi.

Introduction

In the contemporary scenario of ruminant farming, there is a growing emphasis on enhancing production levels while simultaneously ensuring the wellbeing of animals. Gastrointestinal nematode (GIN) control plays a crucial role in addressing both of these essential considerations (Grisi *et al*. 2014).

Animals become infected with GINs mainly through exposure to pastures contaminates with third-stage larvae (L3) (Takeuchi-Storm et al. 2019). The main way to control these parasites is through treating animals with anthelmintic drugs (Vidal et al. 2019; Kaplan 2020), but in many cases, these are administered incorrectly, with excessive and indiscriminate use of therapeutic agents. This increases production costs without achieving effective control of infections (Delgado et al. 2009). In addition, anthelmintics do not act on the free-living stages of nematodes and can leave harmful residues in animal products (SINDAN, 2018).

Protocols for eliminating GINs, such as those of the genera *Haemonchus* and *Cooperia*, consist of use of drug therapies based on synthetic chemical compounds. However, a wide variety of studies have demonstrated helminth resistance to commercially available drugs (Delgado et al. 2009; Vidal et al. 2019; Kaplan 2020). Control with bioproducts is an alternative for managing cattle nematodes in pastures (Braga et al. 2020). The use of biological control methods involving nematophagous fungi has proven to be a safe and viable alternative (Braga et al. 2020; Luns et al. 2018; Vilela et al. 2018) that complements GIN control activities through the action of these fungi in the environment.

Promising results have been obtained with various species of nematophagous fungi, in particular *Duddingtonia flagrans* for controlling helminth larvae and *Pochonia chlamydosporia* with ovicidal action towards controlling helminth eggs (Braga and Araújo 2014; Mendonza-de-Gives et al. 2018; Oliveira et al. 2018; Vieira et al. 2020; Araújo et al. 2021; Mendonza-de-Gives et al. 2022; Oliveira et al. 2022; Araújo 2023).

The use of helminthophagous fungi to complement GIN control is already applicable, as they do not cause undesirable effects in the environment, allowing for a longer residence time. Their environmental action is widely relevant in the GIN control, and they can be dispersed through spores and, in some cases, form chlamydospores, which are highly resistant and can be commercially produced (Delmilho et al 2024).

The *D. flagrans* species used has the characteristic of forming chlamydospores and stands out because of its ability to survive passage through the gastrointestinal tract (GIT) of animals and spread by means of the fecal bolus in the environment (Braga et al. 2020; Vieira et al. 2020; Li et al. 2022; Gronvold et al. 1996; Buzatti et al. 2015), its high production of spores, forming conidia that give rise to hyphae that destroy L3 of nematodes (Fonseca et al. 2022).

Its destructive action occurs through adhesive hyphae that prey on nematodes, and the large quantity of chlamydospores, highly resistant reproductive structures, which, if ingested, travel through the GIT of animals and remain viable when in the feces, colonizing them soon after they are deposited in the soil and preying on nematodes (Calazans et al. 2023).

The fungus *P. chlamydosporia* is important in relation to biocontrol of nematodes (Ayupe, 2020). Its mechanical and enzymatic action destroys helminth eggs by means of appressoria developed from the hyphae, which allow it to colonize

the surface and penetrate, not forming traps (Fonseca et al. 2023) as in the fungus *D. flagrans*.

This fungus develops from hyphae, which facilitate growth and penetration into the surface of the helminth egg (Araújo et al. 2021; Lopez-Llorca et al. 2002). Thus, the development of biological control of GIN is an increasingly attractive option, but requires selection of the most effective fungal isolates or associations of such isolates.

The aim of this study was to evaluate the effects of two commercial bioproducts, one based on *D. flagrans* and the other consisting of an association between *D. flagrans* and *P. chlamydosporia,* for reducing the levels of nematode eggs in animal feces and larval infestations in pastures.

Materials and methods

The study was approved by the Ethics Committee for the Use of Animals (CEUA) of the Federal University of Viçosa (UFV), under reference number 37/2020.

The experiment was conducted on a farm located in the municipality of Abre Campo, state of Minas Gerais, southeastern Brazil, latitude 20° 18' 04 "S, longitude 42° 28' 39" W, from February to October 2021.

Two formulations supplied by GhenVet Saúde Animal (Brazil) were used: a formulation based on the fungus *D. flagrans* known as Bioverm®, which contains 10^6 chlamydospores of the fungus per gram, and an experimental formulation (EF) combining *D. flagrans* and *P. chlamydosporia*, containing 10^6 chlamydospores of each fungus per gram.

The animals were divided according to their average EPG after pilot test, into three groups of six animals (Bioverm® (T1), Experimental Formulation (T2) and Control (C)) each and separated into three 6-ha paddocks of *Brachiaria brizantha*, with *ad libitum* water supply, naturally contaminated with nematode larvae as a result of previous grazing by animals naturally infected with GINs.

Based on this analysis fecal, eighteen male Holstein-Zebu crossbred cattle, with distribution was completely random, only considering average EPG, aged between 12 and 15 months, with an average initial weight of 150 kg, which remained at this average weight. Fecal samples were obtained at the same time of day (1 pm) using the eggs per gram of feces (EPG) every fortnight, accordance with the methods of Gordon and Whitlock 1939 with modifications by Lima 1989 and Dennis et al 1954. After the EPG

fecal culture was carried out using the Roberts and O'Sullivan 1950 technique to identify the genera of helminth larvae.

The experiment lasted nine months (February to October 2021), during which time animal feces and pasture vegetation were collected fortnightly, including the rainy and dry seasons.

In the group that received Bioverm® (T1), each animal was treated with 6 g of the product for every 100 kg of body weight. The product was administered daily, mixed with 1 kg of maize bran. Group T2 received the experimental formulation (mixed with maize meal), also at a daily dose of 6 g/100 kg body weight. In the control group (C), each animal received maize meal daily, without fungal spores. The animals were monitored fortnightly and the dosage of the products was based on body score and average weight, in accordance with previous studies carried out on the same farm, with the same animals, as described by Vieira *et al*. (2020) and Oliveira *et al*. (2022).

Every 15 days from the start of the experiment, two samples of *B. brizantha* grass were collected (0-20 cm and 20-40 cm away from the feces) in the grazing areas of the treated and control groups, at six different points, as described by Raynaud and Gruner (1982), at the same time of day (2 pm). Samples of 500 g of pasture forage were used to recover infective larvae (L3) in accordance with method describe by Lima (1989) and identified using the Roberts and O'Sullivan (1950) technique. The sediment was examined under an optical microscope and the larvae were counted and identified using the criteria established by Keith (1953). The 500 g samples of grass used were placed in an oven at 100 °C to obtain the dry matter. The data obtained were transformed into the number of larvae per kilogram of dry matter.

Climate data on minimum, average and maximum monthly temperatures and monthly precipitation were obtained from the Agricultural Meteorological Monitoring System (Agritempo), available at the website https://www.agritempo.gov.br/agritempo/index.jsp. The averages of EPG, L3 recovered in coproculture and L3 from pasture samples, and weather data during the nine months of the experiment, were converted into monthly values. Then, EPG and L3 data from pasture were subjected to Levene's test for equality of variances, ANOVA and Tukey's test for EPG data, and the chi-square test for L3 in the pasture, in all cases at a significance level of 5%. All tests were carried out using IBM SPSS Statistics 2.0.

Results

Egg counting techniques

The EPG results are presented in Table 1, which shows the average monthly EPG values for the feces in the three groups during the period from February to October 2021.

In the first month (February) of treatment, EPG values did not differ in all groups. In March, EPG values were lower in treatment 1 (6 g of product/100 kg of body weight containing 10^6 chlamydospores per gram of *Duddingtonia flagrans*) than in the control group (C), while in April, May and July, the values were lower in the treatment 1 and treatment 2 groups than in the control (C). In June, August, September and October, there were no differences between the groups.

Table 1. Monthly averages and standard error of the number of eggs per gram of feces (EPG) in treatment 1 (6 g of fungus/100 kg body weight, containing 10^6 chlamydospores per gram of *Duddingtonia flagrans*), treatment 2 (formulation with 6 g of fungus/100 kg body weight, containing 10^6 chlamydospores per gram of *D. flagrans* and *Pochonia chlamydosporia*) and control (C), using the McMaster technique, during the period from February to October 2021, in Abre Campo, Minas Gerais, Brazil.

Month	**Control**	**Treatment 1**	**Treatment 2**
Feb	0.00 ± 0.00**a**	8.33 ± 20.41ª	83.33 ± 204.12**a**
Mar	66.67 ± 66.46ª	0.00 ± 0.00**b**	37.50 ± 49.37**ab**
Apr	95.83 ± 74.86ª	25.00 ± 22.36**b**	8.33 ± 20.41**b**
May	79.17 ± 48.52ª	20.83 ± 24.58**b**	16.67 ± 20.41**b**
Jun	37.50 ± 37.91ª	33.33 ± 25.82ª	20.83 ± 29.23**a**
Jul	62.50 ± 41.08ª	8.33 ± 12.91**b**	12.50 ± 20.92**b**
Aug	70.83 ± 82.79ª	8.33 ± 12.91ª	8.33 ± 12.91**a**
Sep	16.67 ± 25.82ª	33.33 ± 51.64ª	8.33 ± 20.41**a**

Oct	16.67 ± 40.82ª	25.00 ± 61.24ª	25.00 ± 61.24**a**

¹ Data subjected to ANOVA and to Tukey's test, with a significance level of 0.05. Means with different letters on the same line were statistically different, with their respective standard deviations.

Larval recovery techniques

Figure 1 shows the quantities of stage L3 larvae of each genus of GIN recovered from the fecal cultures from animals in T1, T2 and the control group (C).

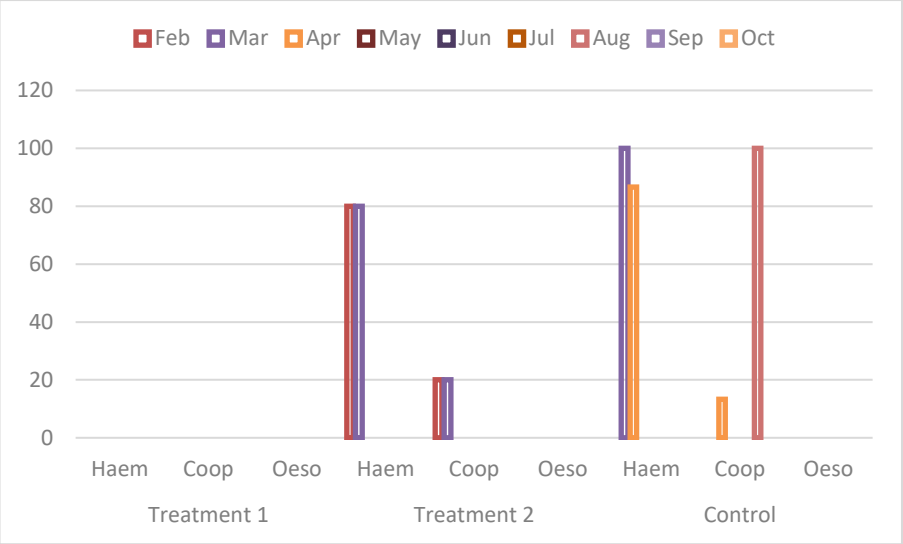


Figure 1. Average values for percentages of infective larvae of the genera *Haemonchus* (Haem), *Cooperia* (Coop) and *Oesophagostomum* (Oeso) recovered from the coprocultures of treatment groups 1 (6 g of fungus/100 kg of body weight, containing 10^6 chlamydospores per gram of *Duddingtonia flagrans*), treatment 2 (formulation with 6 g of fungus/100 kg of body weight, containing 10^6 chlamydospores per gram of *D. flagrans* and *Pochonia chlamydosporia*) and control (C), during the period from February to October 2021 in Abre Campo, Minas Gerais.

The genus *Haemonchus* was predominant, followed by the genus *Cooperia,* in the treatment 2 (EF) and control groups (C). Neither genus was observed in treatment 1 (Bioverm®) after the start of the experiment.

The mean numbers of larvae recovered at both distances from the fecal masses in treatment 1 (6 g of product/100 kg of body weight, containing 10^6 chlamydospores per gram of *Duddingtonia flagrans*), treatment 2 (formulation with 6 g of product/100 kg of

body weight, containing 10^6 chlamydospores per gram of *D. flagrans* and *Pochonia chlamydosporia)* and control (c) differed significantly (p 0.05), as shown in table 2.

Table 2. Mean values and standard errors for numbers of infective larvae recovered per kilogram of dry matter in forage samples from treatment 1 (6 g of fungus/100 kg of body weight containing 10^6 chlamydospores per gram of *Duddingtonia flagrans*), treatment 2 (formulation with 6 g of fungus/100 kg of body weight of *D. flagrans* and *Pochonia chlamydosporia*) and control (C) during the period from February to October 2021, in Abre Campo, Minas Gerais, Brazil.

Larvae per kg of dry matter (0-20 cm)			
Month	**Control**	**Treatment 1**	**Treatment 2**
Feb	2.00	0.00	0.00
Mar	6.00	0.00	3.50
Apr	1.00	0.50	0.00
May	3.00	0.00	0.00
Jun	1.50	0.50	0.50
Jul	0.00	0.00	1.00
Aug	8.00	3.00	0.00
Sep	0.00	5.00	1.00
Oct	0.00	0.00	4.00
Mean	**2.38[a]**	**1.0 b**	**1.11 c**
Larvae per kg of dry matter (20-40 cm)			
Month	**Control**	**Treatment 1**	**Treatment 2**
Feb	1.00	0.50	0.00
Mar	2.00	0.50	0.00
Apr	1.50	12.00	1.50
May	0.50	0.00	0.00
Jun	3.00	0.00	0.00
Jul	1.00	1.50	0.00

Aug	2.50	0.00	0.00
Sep	0.00	0.00	2.00
Oct	7.00	0.00	0.00
Mean	**2.05d**	**1.61b**	**0.38e**

*Different letters on the same line indicate significant differences between the data ($p < 0.05$), according to the chi-square test.

As with the coprocultures, the agents recovered from the pasture belonged predominantly to the genera *Haemonchus* and *Cooperia* in the early stages.

Climatic definitions

Monthly minimum, average and maximum temperatures and monthly precipitation during the experimental period in the municipality of Abre Campo, in the state of Minas Gerais, Brazil, are presented in figure 2.

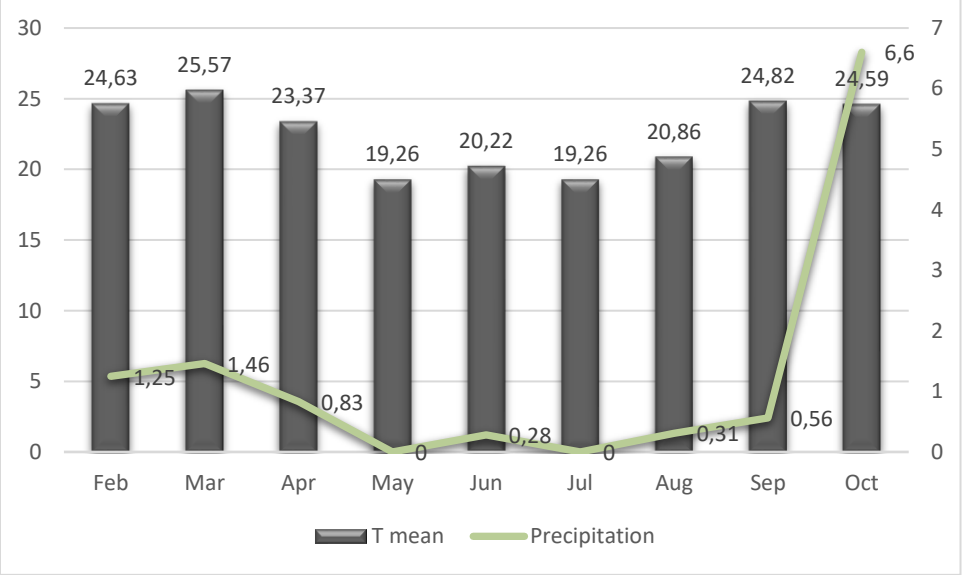


Figure 2. Mean monthly temperatures (T mean) and precipitation (mm^3) from February to October 2021 in the Abre Campo region, Minas Gerais, Brazil.

In 2021, the lowest temperatures were registered in May and July. Monthly average temperatures varied from 19.26 °C in July to 25.57 °C in March. The highest precipitation rate was observed in October (6.60 mm^3), while in the other months rainfall fluctuated between zero in July and 1.46 mm^3 in March.

Discussion

Infection levels were considered low throughout the experiment, this may have been due to the age of the individuals, host resistance mechanisms to parasites or residual control effects on the animals and pasture. During the experimental period, the animal of natural exposure to *B. brizantha* forage without chemical control, natural infection by the most common helminths was observed in this environment was observed. According to epidemiological studies carried out by Kenyon et al. (2009) and Franco et al. (2018), nematodes are found in around 95% of pastures.

According to Kaplan (2020), the effect of anthelmintics eliminates the adult stages and eggs from feces, which was noted in the early analysis. The action of fungi on the environmental life stages of helminths cannot be denied based on the data observed (table 1). The most frequently identified larvae in fecal coprocultures and in the recovery of larvae on pasture were of the genera *Haemonchus* sp. and *Cooperia* sp. (Figure 1 and table 2), which corroborated the findings from earlier research (Mendonza-de-Gives et al. 2018; Oliveira et al. 2018; Vieira et al. 2020; Oliveira et al. 2022).

These genera are highly prevalent within the Brazilian context and their presence gives rise to economic ramifications, including issues such as anemia, nutrient deprivation and even mortality among parasitized animals (Girão et al. 1999; Heckler and Borges, 2016). The findings of the recovery of larvae by the coproculture of feces and pasture differed between treatments, with the recovery of the genera *Haemonchus* spp. and *Cooperia* spp. Being observed in the control and T2, but no in T1.

Parasites of the *Haemonchus* genera are responsible for major losses in cattle farming. They have a great capacity for abomasal lesions due to their blood-spoiling action, wich is relevant to the performance and health of parasitized animals (Marmitt et al. 2020). The *Cooperia* genera influences weight gain, appetite and can lead to diarrhoea, but with less influence (Fonseca et al. 2022).

Similar results were observed for collections between January and July, in wich *Haemonchus* spp. and *Cooperia* spp. were also recovered and there were differences between the groups, with a greater predominance of trichostrongylids (Delmilho et al. 2024). The recovery of larvae fell over the course of the experiment, unlike the study by Delmilho et al. (2024), wich found sudden falls and rises, without uniformity, indicating the lack of predation by the *D. flagrans* fungi, and it is worth noting that they used an underdose of the fungi.

The activity of helminthophagous fungi has been described by numerous authors (Araújo, 2023; Braga and Araújo, 2014; Mendonza-de-Give et al. 2022; Araújo et al. 2021; Carmo et al. 2023). Oral administration of fungi, as in this study, is practical and effective because these fungi can bind to animal feces and can colonize and destroy eggs or capture infective larvae of GINs, acting at an important stage in the life cycle of these parasites (Li et al. 2022; Rodrigues et al. 2021).

In this study, the use of *D. flagrans* fungi or the combined use of *D. flagrans* and *P. chlamydosporia* significantly reduced EPG counts, compared with the control group, during some periods of the experiment: March for treatment 1 and April, May and July for treatments 1 and 2. Fungal control also led to significant reductions in environmental contamination by larvae (Table 2). Similar results were found in previous studies carried out on the same farm, using different fungal bases and forms of application (Vieira et al. 2020; Oliveira et al. 2022). The action of this association of fungi (*D. flagrans* and *P. chlamydosporia*) has also been observed in relation to controlling infective forms of predominantly small strongyle nematodes in horses (Carmo et al. 2023).

This study showed low levels of environmental contamination and the release of eggs and larvae into the pasture, as verified by a previous study carried out in the same location using the control fungi *Arthrobotrys cladodes* and *P. chlamydosporia*, in the form of sodium alginate pellets, which also reduced these parameters, with residual action in the pasture, thus contributing to the low levels of environmental contamination and the release of eggs into the pasture (Vieira et al. 2020).

According to Li et al. (2022), in order to effectively control GINs, it is essential to interfere with their entire life cycle, i.e. to reduce the number of nematodes in animals and on the pasture at the same time. The life cycle of GINs involves the organism of the host animals and the environment (on pastures in extensive grazing systems) (Torres-Acosta and Hoste, 2008). The individual or combined administration of fungi in this study reduced both EPG and L3 in pastures, which indicates the action of these fungi in the environmental phase (free-living phase) of the nematodes. Reducing environmental infestation is a highly desirable factor in parasite control, as it reduces the level of reinfection of animals.

The *D. flagrans* species used has the characteristic of forming chlamydospores and stands out because of its ability to survive passage through the GIT of animals and spread by means of the fecal bolus in the environment (Braga et al. 2020; Vieira

et al. 2020; Li et al. 2022; Gronvold et al. 1996; Buzatti et al. 2015). This fungus has proven to be an excellent biological alternative for controlling infections of animals by nematodes, which explains the reduction in EPG observed over the course of this experiment. The fact that no larvae were recovered from the coprocultures of the group treated with *D. flagrans* spores suggests that this fungus acts on the fecal bolus, to capture and destroy the hatched larvae (Rodrigues et al. 2021; Braga et al. 2008).

D. flagrans has great survival potential in both favourable and unfavourable environmental situations (Mendoza-de-guives et al. 2018), its high production of spores, forming conidia that give rise to hyphae that carry out the destruction of L3 nematodes and chlamydospores, they have a thick wall that gives in the characteristic resistance to survive the environment and the GIT of animals (Fonseca et al. 2023).

Its destructive action occurs by means of adhesive hyphae that prey on nematodes, and the large quantity of chlamydospores, highly resistant reproductive structures, which, if ingested, travel through the TIG of animals and remain viable when in the feces, colonizing them soon after they are deposited in the soil and preying on nematodes (Calazans et al. 2023), the helminths are trapped by hyphae in the shape of three-dimensional nets that penetrate the cuticle and digest even the internal contents of the larva (Fonseca et al 2023). Hence, a commercial product based on chlamydospores of *D. flagrans* (Bioverm®) has been licensed and is marketed in Brazil.

Nematophagous fungi can be distinguished by their ability to generate traps, the hyphae being the format that performs this action (Braga and Araújo 2014). *P. chlamydosporia* acts by means of appressoria developed from the hyphae, which allow it to colonize the surface and penetrate the eggs, but does not form traps (Fonseca et al. 2023) like the fungus *D. flagrans*.

The fungus *P. chlamydosporia* is important in relation to biocontrol of nematodes (Ayupe, 2020). Its mechanical and enzymatic action destroys helminth eggs. From hyphae, which facilitate growth and penetration into the surface of the helminth egg (Araújo et al. 2021; Lopez-Llorca et al. 2002). The EPG counts obtained in this study indicated the action of the fungal association between *D. flagrans* and *P. chlamydosporia*, which may suggest that *P. chlamydosporia* has ovicidal action.

Because they differ in their mechanism of action, the predatory fungus *D. flagrans* and the ovicidal fungus *P. chlamydosporia*, used together, can present synergistic and integrated benefits for the biological control of GINs (Ayupe et al. 2020).

Beyond fungal action, other factors may have influenced the results from this study, such as the height of the grass in the pastures, which varied from 15 cm to 80 cm over the course of the experiment. This variation probably destabilized the microclimate, such that it would become less favorable to the development and survival of the larvae, as well as interfering with protection of the feces and enabling rapid degradation (Rocha et al. 2008).

Climatic conditions are important factors that influence pasture growth and the maintenance of infective larvae (Quadros et al. 2012). In this regard, another factor that influenced the distribution of L3 was the average temperature recorded during the experimental period. According to Heckler and Borges (2016), temperature variations between 13 °C and 26 °C are suitable for maintaining the free-living stages of *Haemonchus* and *Cooperia* larvae. The maximum temperatures recorded were higher than this range in six of the nine months of the experiment (Figure 2).

The high temperatures, together with the lack of rain during the experimental period, especially in the months of May, June, July and August, contributed to reduction in grazing areas and, consequently, increased egg degradation. This reduced the larval load in the pastures in all groups, as indicated by the EPG counts and grazing results in the final months of the experiment.

The presence of water is essential for the migration of L3 from the feces to the pasture (Quadros et al. 2012; Van Dijk and Morgan, 2011). In the present study, although climatic factors may have contributed to the reduction in EPG and L3 infestation in the pastures, the comparison between experimental groups suggests that the fungi *D. flagrans* and *P. chlamydosporia*, alone or in combination, had significant action towards reducing the number of larvae in the pastures and the parasite load of the cattle.

Extensive grazing is the most widespread and productive method for rearing ruminants in Brazil. Therefore, availability of bioproducts for parasite control will contribute towards controlling GINs through their environmental action, thereby minimizing the negative impacts resulting from helminthic infections in animal production systems.

Conclusions

Knowledge about sustainable products is important for enabling rural producers to adopt alternative practices. In this study, use of the nematophagous fungus *D. flagrans* separately or in combination with *P. chlamydosporia* was responsible for reducing egg counts and larval levels in pastures. Thus, application of these bioproducts forms a promising approach towards integrated helminth control among ruminants.

References

ARAÚJO JV. 2023. Advances in the control of helminthiases in domestic animals. Patogens 12: (9), 1107.

ARAÚJO JV. 2014. Diagnóstico das helmintoses. 113 – Ciências Biológicas e da Saúde, Caderno Didático, Viçosa- UFV, 84p.

ARAÚJO JV, BRAGA FR, MENDONZA-DE-GIVES P, PAZ-SILVA A, & VILELA VLR. 2021. Recent advances in the control of helminths of domestic animals by helminthophagous fungi. Parasitology 1:(3), 168-176. https://doi.org/10.3390/parasitologia1030018

AYUPE TH. *Arthrobotrys cladodes* var *macroides* e *Pochonia chlamydosporia* como controladores biológicos de nematoides parasitos gastrintestinais de equinos/ Tiago de Hollanda Ayupe- Viçosa, MG, 2020. 57 f.Orientador: Jackson Victor de Araújo. Tese (doutorado) – Universidade Federal de Viçosa.

BRAGA FR & ARAÚJO JV. 2014. Nematophagous fungi for biological control of gastrointestinal nematodes in domestic animals, Appl Microbiol Biotechnol 98: 71-82.

BRAGA FR, FERRAZ CM, SILVA E & ARAÚJO JV. 2020. Efficiency of the Bioverm (*Duddingtonia flagrans*) fungal formulation to control in vivo and in vitro of *Haemonchus contortus* and *Strongyloides papillosus* in sheep. 3 Biotech: 10, 62.

BRAGA FR, ARAÚJO JV, CAMPOS AK, SILVA AR, ARAÚJO JM, CARVALHO RO, CORRÊA DN & PEREIRA CAJ. 2008. In vitro evaluation of the effect of the nematophagous fungi *Duddingtonia flagrans*, *Monacrosporium sinense* and *Pochonia chlamydosporia* on *Schistosoma mansoni* eggs. World J Microbiol Biotechnol 24: 2713–2716.

Brasil. Ministério da Agricultura, Pecuária e Abastecimento (MAPA). *Regulamento Técnico para Licenciamento e/ou Renovação de Licença de Produtos Antiparasitários de Uso Veterinário.* Portaria nº 48, de 12 de maio de1997. Diário Oficial da União de 16/05/1997, Seção 1, Página 10165.

BUZATTI A, SANTOS PC, FERNANDES MAM, YOSHITANI UY, SPRENGER LK, DOS SANTOS CD & MOLENTO MB. 2015. *Duddingtonia flagrans* in the control of gastrointestinal nematodes of horses. Exp Parasitol 159: 1–4.

CALAZANS FB et al. 2023. Avaliação do fungo Duddingtonia flagrans e hipoclorito de sódio a 5% sobre a eclodibilidade de ovos de ciatostomínaeos. Rev Med Vet 17:4, 225-229.

CARMO TA, MENA MO, CIPRIANO IA, DE FAVARE GM, GUELPA GJ, PINTO SC, AMARANTE AFT, ARAÚJO JV & SOUTELLO RVG. 2023. Biological controlo f gastroinstestinal nematodes in horses fed with grass in association with nematophagus fungi *Duddingtonia flagrans* and *Pochonia chlamydosporia*. Bio control 182: 105-219.

DELGADO FEF, LIMA WS, CUNHA AP, BELLO ACPP, DOMINGUES LN, WANDERLEY RPB, LEITE PVB & LEITE RC. 2009. Verminoses dos bovinos: percepção de pecuaristas em Minas Gerais, Brasil. Rev Bras Parasitol Vet 18: 29–33. doi:10.4322/rbpv.01803005.

DELMILHO G, BOHLAND E, STEPHANIE N, VAZ CCP, ALVAREZ LR, COSTA RLD. 2024. Evaluation of the supply of *Duddingtonia flagrans* for the control of gastrointestinal parasites in sheep. An Acad Bras Ciênc 96: (1) 1-13. Doi: https://doi.org/10.1590/00013765202420220940

DENNIS WR, STONE WM & SWANSON LE. 1954. A new laboratory and field diagnostic test for fluke ova, in feces. J Amer Vet Med. Am 124: 47-50.

FONSECA JS, FERREIRA VM, FREITAS SG, VIEIRA IS & ARAUJO JV. 2022. Efficacy of a Fungal Formulation with the Nematophagous Fungus Pochonia chlamydosporia in the Biological Control of Bovine Nematodiosis. Pathogens 11:6, 695; https://doi.org/10.3390/pathogens11060695

FONSECA JS, ALTOÉ LSC, CARVALHO LM, SOARES FEF, BRAGAFR & ARAÚJO JV. 2023. Nematophagous fungus Pochonia chlamydosporia to control parasitic diseases in animals. App Microb Bio 107: 3859-3868.

FRANCO B, EUSÉ LA, SAGUES MF, IGLESIAS LE, FERNANDEZ AS, ZEGBI S, GUERREIRO I & SAMUELL CA. 2018. Predatory effect of *Duddingtonia flagrans* on infective larvae of gastro-intestinal parasites under sunny and shaded conditions. Exp Parasitol 193: 27–32.

GIRÃO ES, LEAL JA, GIRÃO RN & MEDEIROS LP. 1999. Verminose bovina. Teresina: Embrapa Meio Norte. Documento 41, 30p.

GRISI L, LEITE RC, MARTINS JRS, BARROS ATM, ANDREOTTI R, CANÇADO PHD, DE LEON AAP, PEREIRA JB & VILLELA HS. 2014. Reassessment of the potential economic impact of cattle parasites in Brazil. Rev Bras Parasitol Vet *23*: 150–156. doi:10.1590/S1984-29612014042.

GORDON HM & WHITLOCK HV. 1939. A new technique for counting nematode eggs in sheep feces. J Sci Ind Res 12: 50-52.

GRONVOLD J, HENRIKSEN SA, LARSEN M, NANSEN P & WOLSTRUP J. 1996. Biological control-Aspects of biological control-With special reference to arthropods, protozoans and helminths of domesticated animals. Vet Parasitol 64: 47–68.

HECKLER RP & BORGES FA. 2016. Climate variations and the environmental population of gastrointestinal nematodes of ruminants. Nematoda 3.

KAPLAN RM. 2020. Biology, Epidemiology, Diagnosis, and Management of Anthelmintic Resistance in Gastrointestinal Nematodes of Livestock. Vet Clin North Am Food Anim Pract *36*: 17–30. doi: https://doi.org/10.1016/j.cvfa.2019.12.001.

KEITH RK. 1953. The differentiation on the infective larvae of some common nematode parasites of cattle. Austr Jour Zool 1: 223-235.

LIMA WS. 1989. Dinâmica das populações de nematoides parasitos gastrintestinais em bovinos de corte, alguns aspectos da relação parasito-hospedeiro e do comportamento dos estádios de vida livre na região do Vale do Rio Doce, MG, Brasil. Thesis (Ph. Doctor). Universidade Federal de Minas Gerais, MG, Brasil.

KENYON F ET AL. 2009. The role of targeted selective treatments in the development of refugia-based approaches to the control of gastrointestinal nematodes of small ruminants. Vet Parasitol 164: 3–11.

LI S, WANG D, GONG J & ZHANG Y. 2022. Individual and Combined Application of Nematophagous Fungi as Biological Control Agents against Gastrointestinal Nematodes in Domestic Animals. J Pathog 11: 172.

LIMA WS & GUIMARÃES MP. 1992. Comportamento das infecções helminticas em vacas de rebanho de corte durante a gestação e lactação. Arq Bras Med Vet Zoo 44: (5) 387-396.

LOPEZ-LLORCA LV, OLIVARES-BERNABEU C, SALINAS J, JANSSON HB & KOLATTUKUDY PE. 2002. Pre-penetration events in fungal parasitism of nematode eggs. Mycol Res 106: 499–506.

LUNS FD, ASSIS RCL, SILVA LPC, FERRAZ CM, BRAGA FR & ARAÚJO JV. 2018. Coadministration of nematophagous fungi for biological control over nematodes in bovine in the South-eastern Brazil. Biomed Res Int 1-6.

MARMITT IVP, SEDREZ FS, FÉLIX SR, NIEDERMEYER F, CHAGAS BC, NIZOLI LQ. 2020. Estudo transversal da ocorrência de *Haemonchus* spp. em bovinos de corte submetidos a diferentes sistemas de terminação. Braz J of Develop 6: 8, p.58624- 58633.

MENDONZA-DE-GIVES P, LÓPEZ-ARELLANO MA, AGUILAR-MARCELINO L, OLAZARAN-JENKINS S, REYES-GUERRERO D, RAMÍREZ-VARGAS G & VEGA-MURLLO V. 2018. The nematophagous fungus *Duddingtonia flagrans* reduces the

gastrointestinal parasitic nematode larvae population in faeces of orally treated calves maintained under tropical conditions - Dose/response assessment. Vet Parasitol 263, 66-72.

MENDONZA-DE-GIVES P, BRAGA FR & ARAÚJO JV. 2022. Nematophagous fungi, an extraordinary tool for controlling ruminant parasitic nematodes and other biotechnological applications. Biocontrol Sci Technol 32: 777-793. https://doi.org/10.1080/09583157.2022.2028725.

OLIVEIRA IC, VIEIRA IS, CARVALHO LM, CAMPOS AK, FREITAS SG, ARAÚJO JM, BRAGA RB & ARAÚJO JV. 2018. Reduction of bovine strongilides in naturally contaminated pastures in the southeast region of Brazil. Exp Parasitol 194: 9-15.

OLIVEIRA IC, VIEIRA IS, FREITAS SG, CAMPOS AK, & ARAÚJO JV. 2022. *Monascrosporium sinense* e *Pochonia chlamydosporia* para o controle biológico de larvas infectantes bovinas em pastagem de *Brachiaria brizantha*. Bio Control 171.

QUADROS DG, SOBRINHO AGS, RODRIGUES LRA, OLIVEIRA GP, XAVIER CP & ANDRADE AP. 2012. Effect of three species of forage grasses on pasture structure and vertical distribution of infective larvae of gastrointestinal nematodes of sheep. Ci Anim Bras Goiânia 13: 139-144.

RAYNAUD JP & GRUNER L. 1982. Feasibility of herbage sampling in large extensive and avaibility of cattle nematode in mountain pastures. Vet Parasitol 10: 57-64.

ROBERTS FFS & O'SULLIVAN PJ. 1950. Methods for egg counts and larval culture for strongyles infesting the gastrointestinal tract of cattle. Aust J Agric Res 1:99-102.

ROCHA RA, ROCHA GP, BRICARELLO PA & AMARANTE AFT. 2008. Recovery of *Trichostrongylus colubriformis* infective larvae from three grass species contaminated in summer. Rev Bras Parasitol Vet 17: 227-234.

RODRIGUES JA, ROQUE FL, ALVARES FBV, DA SILVA ALP, DE LIMA EF, FILHO GMD, FEITOSA TF, ARAÚJO FR & VILELA VLR. 2021. Efficacy of a commercial fungal formulation containing *Duddingtonia flagrans* (Bioverm (R)) for controlling bovine gastrointestinal nematodes. Rev Bras Parasitol Vet 28,30: (2) e026620. DOI: 10.1590/S1984-29612021025

Sindicato Nacional da Indústria de Produtos para saúde animal. *Mercado nacional de produtos para saúde animal.* 2018. Available online: http://www.sindan.org.br/mercado-brasil-2018/ (accessed on Jun 24, 2024).

TAKEUCHI-STORM N ET AL. 2019. Parasite control in organic cattle farming: Management and farmers' perspectives from six European countries. Vet Parasitol Reg Stud Reports 18.

TORRES-ACOSTA JFL & HOSTE H. 2008. Alternative or improved methods to limit gastro intestinal parasitism in grazing sheep and goats. Small Ruminant Res 77: 159-173.

VAN DIJK J & MORGAN ER. 2011. The influence of water on the migration of infective trichostrongyloid larvae onto grass. Parasitol 138: 780-788.

VIDAL MLB, VIANA MVG, ITO M, TRIVILIN LO & MARTINS IVF. 2019. Anti-helmínticos de importância veterinária no Brasil. In *Tópicos especiais em ciência animal VIII*; CAUFES: Alegre pp. 273–297.

VIEIRA IS, OLIVEIRA IC, FREITAS SG, CAMPOS AK & ARAÚJO JV. 2020. *Arthrobotrys cladodes* and *Pochonia chlamydosporia* in the biological control of nematodiosis in extensive bovine production system. Parasitol 147: 1-24.

VILELA VLR, FEITOSA TF, BRAGA FR, VIEIRA VD, LUCENA SC & ARAÚJO JV. 2018. Control of sheep gastrointestinal nematodes using the combination of *Duddingtonia flagrans* and levamisole hydrochloride 5%. Rev Bras Parasitol Vet 27: 23-31.

WOOD JB ET AL.*1995.* World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). Vet Parasitol 58: 181-213.

5. CAPÍTULO 2

GROWTH OF NEMATOPHAGOUS FUNGI IN THE PRESENCE OF ANTHELMINTICS DRUGS

GROWTH OF NEMATOPHAGOUS FUNGI IN THE PRESENCE OF ANTHELMINTICS DRUGS

Abstract

This study investigated the activity of commercial anthelmintics for ruminants based on levamisole hydrochloride 7.5% (Ripercol®), abamectin solution 1% (Calbos®) and albendazole sulphoxide solution 15% (Agebendazole®) on the growth of the nematophagous fungi: *Arthrobotrys musiformis*, *Duddingtonia flagrans* and *Pochonia chlamydosporia*. The formulations were diluted in proportional parts, creating four levels of dilution (DL1, DL2, DL3 and DL4). For albendazole and abamectin the concentrations for each diluted solution were 1, 10, 100, 1000 ppm and for levamisole the concentrations were 2.23, 22.3, 223 and 2230 ppm. The treatments were accompanied by: negative control (NC) – culture medium only (PDA 2%); positive control (PC) – culture medium with fungal strain; and water control (WC). The response for each species of fungus was carried out using a factorial scheme, with three drugs and four dilutions, tested with three repetitions. The results showed that the fungi *A. musiformis* and *P. chlamydosporia* suffered less influence on their growth under the action of the anthelmintics, unlike *D. flagrans* which suffered greater inhibition of its growth. The anthelmintic albendazole at concentrations of 1 and 10 ppm had a greater influence on fungal growth. Understanding the viability of these associations is fundamental to establishing protocols aimed at reducing the incidence of worms in cattle farming.

Keywords: Antiparasitic; *Arthrobotrys musiformis;* Biological control; *Duddingtonia flagrans;* Helminthophagous fungi; *Pochonia chlamydosporia.*

Introduction

The problems caused by worms in cattle farming are significant, creating a need to improve the effectiveness of current control protocols Fonseca et al. 2022. One promising approach is using nematophagous fungi to reduce the parasite population, exploring their natural antagonismo Araújo 2023; Melo et al 2023. Nematophagous fungi can present synergism with pharmaceutical agents, promoting their action over helminth eggs and larvae. This approach can be used alongside chemical control, especially against the infective forms present in feces and in the environment Araújo et al 2009; Braga et al 2008.

Integrating different methods of control can be an advantageous strategy; however, to better explore this strategy, it is important to understand the effect of anthelmintics on the fungi activity. This issue is described by Neves et al 2001, and Alizadeh et al 1560, who indicate that synthetic products, such as anthelmintics, can inhibit the development of nematophagous fungi. The use of these products in conjunction with fungi can interfere with, and even inhibit, the growth and sporulation of these microorganisms, resulting in a reduction in their effectiveness and performance. This issue is of crucial importance in the context of using nematophagous fungi to control gastrointestinal parasites.

Chemical control (synthetic) of helminthiases is commonly carried out following the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) Powers et al 1982; Wood et al. 1995. The elimination of anthelmintic residues in animal feces is essential, since the fecal bolus is where the main action of nematophagous fungi takes place Vieira et al 2020; therefore, residual components may have an effect over the fungi in the fecal bolus and in the soil.

As demonstrated by Navrátilová et al 2023, who carried out a study with albendazole (ABZ) and found two main metabolites of ABZ, ABZ-sulfoxide (active anthelmintic) and ABZ-sulfone (inactive), which persisted in the soil (up to 25 cm from the feces) and in the plants for three months after the end of the experimental period.

Junco et al. 2021 described the adverse effects of macrocyclic lactones, at concentrations in which they are eliminated in the feces of domestic animals, for different fungi species which colonize their manure.

It is important to better understand integrated actions, such as the use of nematophagous fungi, which have become increasingly important. This topic has been increasingly studied and several recent studies have affirmed the feasibility of its application Ayupe 2020; Vieira 2020; Araújo 2023, giving rise to the need to know the relationship between the available forms of control and the feasibility of their adoption in an integrated manner.

The relative scarcity of recent studies on the compatibility between specific nematophagous fungi and anthelmintics with *in vitro* effects Wang et al 2021; Zegbi et al 2024, together with the need to standardize methods for *in vitro* and then *in vivo* tests, motivated this research, with the objective of investigating the effect of drugs in the anthelmintic treatment of ruminants over the growth of nematophagous fungi used in the biological control of helminths.

Material and Methods

Fungal isolates

Three species of nematophagous fungi were used, isolated and identified according to the procedure carried out by Araújo et al 1993, the species being *Arthrobotrys musiformis* (isolate-I144), *Duddingtonia flagrans* (isolate-AC001) and *Pochonia chlamydosporia* (isolate-VC04), available from the nematophagous fungi collection of the Parasitology Laboratory of the Veterinary Department of the Federal University of Viçosa – Brazil.

The original fungi were stored in test tubes containing 2% corn-meal agar (CMA), medium in a mycotheque, with dark environment at 4°C. The fungi were transferred to Petri dishes (9 cm of diameter) containing 2% agar medium (AA) and grown for 7 days in a BOD (Biological Oxygen Demand) chamber at 26 ºC in the absence of light.

Experimental Test

The response for each species of fungus was studied using experimental trials that followed a 3×4 factorial scheme, with three drugs and four dilution levels, tested in a completely randomized experimental design with three repetitions.

The three drugs tested in this study were: levamisole hydrochloride solution 7.5% (Ripercol®), abamectin solution 1% (Calbos®) and albendazole sulphoxide solution 15% (Agebendazole®), in commercial formulations.

The susceptibility test was carried out using the microdilution technique in accordance with reference document M38-A2, adapted for anthelmintic drugs Rex et al 2008. The solutions were diluted with distilled water and mixed with potato dextrose agar (PDA 2%) and the anthelmintics, with a mixture of 10 mL of each drug (levamisole, abamectin and albendazole) in 100 mL of the culture medium.

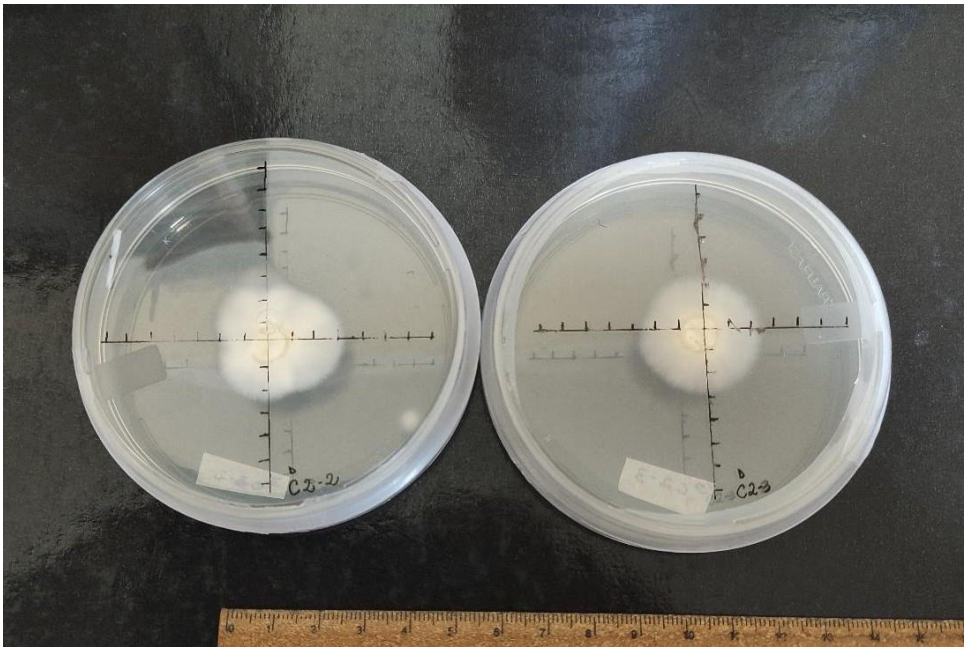
Albendazole and abamectin were diluted to 1, 10, 100 and 1000 parts per million (ppm) and levamisole hydrochloride to 2, 23, 22.3, 223 and 2230 ppm, forming four dilution levels for each drug: DL1 (factor: 1), DL2 (factor: 10), DL3 (factor: 100) and DL4 (factor: 1000) based on the methodology adapted from CLSI Rex et al 2008. Control treatments were established: negative control (NC), containing only the

presence of the culture medium (2% PDA); positive control (PC), formed by the presence of the culture medium and the fungal strain; and water control (WC), by adding 10 mL of distilled water to 100 mL of 2% PDA medium, in order to guarantee the reproducibility and accuracy of the test.

A total of 135 Petri dishes were prepared with samples of the three fungi subdivided into plots of 4 dilutions and individualized controls for each fungus, to analyze their mycelial growth for seven days. Thus, the experimental plots consisted of one Petri dish prepared with its respective treatment.

The Petri dishes were placed in BOD chambers and kept in the dark at 27ºC, with fungal growth being daily monitored. Measurements were taken to check colony growth in two perpendicular directions, in centimeters (cm), until maximum growth was reached in each Petri dish Cadioli et al 2007. Mycelial growth was chosen for analysis since it is related to the fungi propagation and survival factor. The analysis technique is shown in figure 1.

Figure 1. Trial registry showing the analysis of growth of nematophagous fungi being assessed during the seven days of observation in 2% Potato Dextrose Agar.



Source: Personal archive

Daily evaluations were carried out over a period of seven days, at the same period of the day, with each experimental plot recorded individually. At the end of the mycelial growth assessment period, the data was tabulated and subjected to analysis of variance and, according to the significance of the sources of variation, the means were compared using the

Tukey test, both at 5% probability. The analyses were carried out using the SISVAR statistical software, version 5.6 Ferreira 2011.

Results

The obtained results were represented as mean of growth from each trial carried out with the fungi *Arthrobotrys musiformis, Duddingtonia flagrans* and *Pochonia chlamydosporia,* which were subjected to different concentrations of anthelmintics (abamectin, albendazole and levamisole) and evaluated for seven days to check their effect on mycelial growth. These results are presented in the following tables, differentiating the fungi as *A. musiformis* (Table 1), *D. flagrans* (Table 2) and *P. chlamydosporia (*Table 3).

There was a significant effect of the interaction between the sources of variation (drugs and dilutions), showing that the effect of dilution and the type of drug acted together to determine the growth of *A. musiformis,* only initially (up to day 3). This interaction between factors was not observed after the fourth day, after which there was no significant effect between drugs or dilutions until the seventh day. On the seventh day, fungal growth was not significantly influenced by dilution, but was influenced by the type of drug used (Table 1).

In Table 1, showing the growth activity of the *A. musiformis* fungus, on day 1 there were no significant differences among means for any of the anthelmintics or their dilutions. On day 2, as on day 1, there were no significant differences among any of the anthelmintics. On day 3, unfolding the interaction showed that the dilution only had an effect on the anthelmintic albendazole, for which the dilution at 0.1 PPM caused the lowest fungal growth. On that same day, a differentiation was noticed among the anthelmintics in DL1 and DL3, for which the lowest fungal growth was obtained with the use of albendazole (DL1) and albendazole and levamisole (DL3). On days 4, 5 and 6, growth again reached similar levels and there were no significant differences among means for any of the anthelmintics. At the end of the evaluation period (day 7), the use of albendazole allowed greater fungal growth, while abamectin resulted in the lowest mycelial growth and levamisole presented an intermediate effect between the other drugs.

Table 1. Mean mycelial growth, in centimeters, for *Arthrobotrys musiformis* (isolate-I144) under the effect of the anthelmintics: abamectin, levamisole and albendazole; for 7 days, at dilutions of 1 ppm (DL1) 10 ppm (DL2), 100 ppm (DL3), 1000 ppm (DL4) for albendazole and abamectin of 2, 23 ppm (DL1), 22.3 ppm (DL2), 223 ppm (DL3) and 2230 ppm (DL4), in 2% potato dextrose agar culture medium.

Anthelmintic	DL1	DL2	DL3	DL4	Mean

Day 1					
Abamectin	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0,00±0,00
Albendazole	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0,00±0,00
Levamisole	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0,00±0,00
Mean	0,00±0,00	0,00±0,00	0,00±0,00	0,00±0,00	
Day 2					
Abamectin	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0,00±0,00
Albendazole	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0,00±0,00
Levamisole	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0,00±0,00
Mean	0,00±0,00	0,00±0,00	0,00±0,00	0,00±0,00	
Day 3					
Abamectin	0.30±0.00 Aa	0.32±0.08 Aa	0.43±0.06 Aa	0.30±0.00 Aa	0,34±0,07
Albendazole	0.10±0.00 Bb	0.30±0.00 Aa	0.22±0.10 ABb	0.27±0.06 Aa	0,22±0,09
Levamisole	0.30±0.10 Aa	0.30±0.00 Aa	0.30±0.10 Ab	0.30±0.00 Aa	0,30±0,06
Mean	0,23±0,11	0,31±0,04	0,32±0,12	0,29±0,03	
Day 4					
Abamectin	0,60±0,10	0,55±0,05	0,87±0,29	0,63±0,10	0,66±0,19 a
Albendazole	0,77±0,21	0,77±0,06	0,80±0,20	0,67±0,06	0,75±0,14 a
Levamisole	0,72±0,06	0,68±0,08	0,70±0,09	0,70±0,10	0,70±0,07 a
Mean	0,69±0,14 A	0,67±0,11 A	0,79±0,19 A	0,67±0,08 A	
Day 5					
Abamectin	1,40±0,13	1,35±0,13	1,63±0,32	1,40±0,10	1,45±0,20 a
Albendazole	1,45±0,05	1,50±0,00	1,68±0,16	1,60±0,20	1,56±0,15 a
Levamisole	1,53±0,06	1,55±0,09	1,47±0,06	1,45±0,23	1,50±0,12 a
Mean	1,46±0,10 A	1,47±0,12 A	1,59±0,21 A	1,48±0,18 A	
Day 6					
Abamectin	2,35±0,15	2,30±0,20	2,63±0,32	2,20±0,20	2,37±0,26 a
Albendazole	2,40±0,10	2,32±0,16	2,55±0,13	2,37±0,29	2,41±0,18 a
Levamisole	2,50±0,00	2,50±0,10	2,50±0,00	2,40±0,17	2,48±0,10 a
Mean	2,42±0,11 A	2,37±0,17 A	2,56±0,18 A	2,32±0,22 A	
Day 7					
Abamectin	3,17±0,29	3,10±0,00	3,23±0,21	3,00±0,00	3,13±0,18 b

Albendazole	3,07±0,06	3,33±0,15	3,50±0,00	3,33±0,29	3,31±0,22 a
Levamisole	3,27±0,21	3,50±0,00	3,23±0,06	3,13±0,23	3.28±0.19 ab
Mean	3,17±0,20 A	3,31±0,19 A	3,32±0,17 A	3,16±0,24 A	

Means followed by the same uppercase letter in each row and lowercase letter in each column do not differ statistically by the Tukey test, at 5% probability.

There was a significant effect of the interaction to determine the fungal growth of *D. flagrans*. The breakdown of this interaction for each day is shown in Table 2. On days 1 and 2, there were no significant effects of the anthelmintics or their dilutions. On day 3, the dilutions were only statistically different for levamisole, for which DL4 allowed greater mycelial growth.

On that day, for the dilutions DL2 and DL3, the use of albendazole caused greater fungal growth than the other drugs. On day 4, the same response was maintained in relation to the dilutions of each drug; however, it was possible to observe greater and similar fungal growth with the use of albendazole and abamectin for DL1, DL2 and DL3. Only in DL4, levamisole allowed greater fungal growth than abamectin. On day 5, the effect of the dilutions remained the same, with only levamisole having an effect and DL4 being the dilution with the greatest mycelial growth. In DL2, albendazole alone allowed the greatest fungal growth; while in DL4, the greatest growth was obtained with the use of levamisole. On day 6, the effect of dilutions continued to be significant only for levamisole, allowing greater growth with levels closer to LD4. At LD1, Levamisole caused greater restriction to fungal growth; however, at LD4, this drug caused the greatest growth alone. At the end of the evaluation period (day 7), the effect of the dilutions remained significant only for levamisole, with lower growths using DL1. At this dilution level, abamectin and albendazole allowed greater mycelial growths. For the other dilutions, the fungal growths did not differ statistically from each other regardless of the anthelmintic used.

Table 2. Mean mycelial growth, in centimeters, for *Duddingtonia flagrans* (isolate AC001) under the effect of the anthelmintics: abamectin, levamisole and albendazole; for 7 days, at dilutions of 1 ppm (DL1) 10 ppm (DL2), 100 ppm (DL3), 1000 ppm (DL4) for albendazole and abamectin of 2, 23 ppm (DL1), 22.3 ppm (DL2), 223 ppm (DL3) and 2230 ppm (DL4), in 2% potato dextrose agar culture medium.

Anthelmintic	DL1	DL2	DL3	DL4	Mean
Day 1					

Abamectin	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0,00±0,00
Albendazole	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0,00±0,00
Levamisole	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0,00±0,00
Mean	0,00±0,00	0,00±0,00	0,00±0,00	0,00±0,00	
Day 2					
Abamectin	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0,00±0,00
Albendazole	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0,00±0,00
Levamisole	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0,00±0,00
Mean	0,00±0,00	0,00±0,00	0,00±0,00	0,00±0,00	
Day 3					
Abamectin	0.27±0.06 Aa	0.33±0.12 Ab	0.53±0.06 Aab	0.43±0.12 Aa	0,39±0,13
Albendazole	0.50±0.00 Aa	0.60±0.17 Aa	0.77±0.25 Aa	0.50±0.00 Aa	0,59±0,17
Levamisole	0.30±0.00 Ba	0.20±0.00 Bb	0.33±0.15 Bb	0.67±0.15 Aa	0,38±0,21
Mean	0,36±0,11	0,38±0,20	0,54±0,24	0,53±0,14	
Day 4					
Abamectin	0.68±0.08 Aab	0.67±0.12 Aab	0.87±0.13 Aab	0.67±0.03 Ab	0,72±0,12
Albendazole	0.75±0.09 Aa	0.87±0.08 Aa	0.97±0.21 Aa	0.83±0.06 Aab	0,85±0,13
Levamisole	0.50±0.00 Bb	0.60±0.00 Bb	0.67±0.08 Bb	1.02±0.18 Aa	0,70±0,22
Mean	0,64±0,13	0,71±0,14	0,83±0,18	0,84±0,18	
Day 5					
Abamectin	1.27±0.15 Aa	1.32±0.16 Aab	1.40±0.10 Aa	1.22±0.10 Ab	1,30±0,13
Albendazole	1.33±0.06 Aa	1.50±0.10 Aa	1.37±0.21 Aa	1.35±0.13 Aab	1,39±0,14
Levamisole	1.20±0.26 Ba	1.07±0.06 Bb	1.23±0.15 Ba	1.60±0.20 Aa	1,28±0,26
Mean	1,27±0,17	1,29±0,21	1,33±0,16	1,39±0,21	
Day 6					
Abamectin	1.87±0.23 Aa	1.90±0.10 Aa	1.95±0.09 Aa	1.72±0.10 Ab	1,86±0,15
Albendazole	1.93±0.12 Aa	1.95±0.09 Aa	2.00±0.20 Aa	1.95±0.09 Aab	1,96±0,11
Levamisole	1.37±0.32 Cb	1.62±0.13 BCa	1.83±0.18 Aba	2.17±0.15 Aa	1,75±0,35
Mean	1,72±0,34	1,82±0,18	1,93±0,16	1,94±0,22	
Day 7					
Abamectin	2.47±0.15 Aa	2.50±0.10 Aa	2.60±0.20 Aa	2.40±0.10 Aa	2,49±0,14
Albendazole	2.53±0.06 Aa	2.47±0.42 Aa	2.58±0.18 Aa	2.40±0.17 Aa	2,50±0,22

Levamisole	1.57±0.60 Bb	2.13±0.31 ABa	2.63±0.31 Aa	2.73±0.23 Aa	2,27±0,59
Mean	2,19±0,56	2,37±0,32	2,61±0,20	2,51±0,23	

Means followed by the same uppercase letter in each row and lowercase letter in each column do not differ statistically by the Tukey test, at 5% probability.

Table 3 shows the growth of the fungus *P. chlamydosporia* (isolate VC04), there was no significant effect of the interaction between drugs and dilutions, nor of the dilutions alone. The differences observed from day 3 onwards were determined only by the effect of the type of drug (Table 3).

On days 1 and 2, there were no significant differences among means for any of the anthelmintics. On day 3, abamectin allowed greater mycelial growth, while levamisole caused more limitations over the growth. On days 4 and 5, the same behavior was observed, with greater mycelial growth being obtained with the use of abamectin, followed by levamisole. The lowest mycelial growth was observed with the use of albendazole. For days 6 and 7, abamectin allowed the greatest fungal growth, while the other anthelmintics did not differ from each other.

Table 3. Mean mycelial growth, in centimeters, for *Pochonia chlamydosporia* (isolate VC04) under the effect of the anthelmintics: abamectin, levamisole and albendazole; for 7 days, at dilutions of 1 ppm (DL1) 10 ppm (DL2), 100 ppm (DL3), 1000 ppm (DL4) for albendazole and abamectin of 2, 23 ppm (DL1), 22.3 ppm (DL2), 223 ppm (DL3) and 2230 ppm (DL4), in 2% potato dextrose agar culture medium.

Anthelmintic	DL1	DL2	DL3	DL4	Mean
Day 1					
Abamectin	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0,00±0,00
Albendazole	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0,00±0,00
Levamisole	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0,00±0,00
Mean	0,00±0,00	0,00±0,00	0,00±0,00	0,00±0,00	
Day 2					
Abamectin	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0,00±0,00
Albendazole	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0,00±0,00
Levamisole	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0.00±0.00 Aa	0,00±0,00
Mean	0,00±0,00	0,00±0,00	0,00±0,00	0,00±0,00	
Day 3					

Abamectin	0,13±0,14	0,12±0,08	0,27±0,06	0,27±0,21	0,20±0,14 a
Albendazole	0,17±0,06	0,08±0,03	0,13±0,08	0,15±0,09	0.13±0.07 ab
Levamisole	0,13±0,14	0,08±0,03	0,08±0,03	0,05±0,00	0,09±0,07 b
Mean	0,14±0,11 A	0,09±0,05 A	0,16±0,10 A	0,16±0,15 A	
Day 4					
Abamectin	0,33±0,14	0,42±0,14	0,30±0,00	0,48±0,28	0,38±0,16 a
Albendazole	0,20±0,00	0,15±0,00	0,15±0,05	0,25±0,13	0,19±0,07 b
Levamisole	0,23±0,08	0,33±0,21	0,40±0,26	0,10±0,00	0.27±0.19 ab
Mean	0,26±0,10 A	0,30±0,17 A	0,28±0,17 A	0,28±0,23 A	
Day 5					
Abamectin	0,85±0,35	0,73±0,12	0,67±0,12	1,23±0,86	0,87±0,46 a
Albendazole	0,45±0,09	0,43±0,06	0,50±0,00	0,47±0,15	0,46±0,08 b
Levamisole	0,70±0,26	0,90±0,46	0,93±0,31	0,22±0,03	0.69±0.40 ab
Mean	0,67±0,28 A	0,69±0,31 A	0,70±0,25 A	0,64±0,63 A	
Day 6					
Abamectin	1,03±0,40	1,27±0,46	2,17±1,26	1,37±0,78	1,46±0,82 a
Albendazole	0,93±0,51	0,50±0,00	0,67±0,29	0,80±0,30	0,73±0,33 b
Levamisole	0,87±0,12	1,27±0,31	1,22±0,20	0,22±0,03	0,89±0,47 b
Mean	0,94±0,34 A	1,01±0,47 A	1,35±0,93 A	0,79±0,65 A	
Day 7					
Abamectin	1,60±0,78	1,77±0,90	2,50±1,50	1,93±0,86	1,95±0,96 a
Albendazole	1,37±0,47	0,73±0,40	0,73±0,25	1,03±0,50	0,97±0,45 b
Levamisole	1,23±0,15	1,50±0,26	1,83±1,04	0,22±0,03	1,20±0,78 b
Mean	1,40±0,49 A	1,33±0,69 A	1,69±1,20 A	1,06±0,90 A	

Means followed by the same uppercase letter in each row and lowercase letter in each column do not differ statistically by the Tukey test, at 5% probability.

Discussion

The results presented for the exposure of the three nematophagous fungi analyzed in this study, *A. musiformis*, *D. flagrans* and *P. chlamydosporia*, considering dilutions with different concentrations of anthelmintics (abamectin, albendazole and levamisole), showed that all fungi only had mycelial growth past the second day.

It is important to emphasize that the mycelial growth is involved in the proliferation and survival of the fungi Dackman 1992. The aim of this study was to analyze possible interventions in fungal growth and development and, consequently, in their predation capacity. According to Persson and Jansson 1997, fungi have two phases: saprophytic and parasitic, and they have the ability to change due to environmental stimulus, being able to adapt and carry out the necessary activity defined for the situation, *i.e.* nourish and multiply or carry out captures and seizures.

For the fungus *Arthrobotrys musiformis*, there was an increase in mycelial growth at all anthelmintic concentrations from the third day onwards, with some variations among concentrations for each period. The growth pattern varied according to the type of anthelmintic; however, there were not many significant differences in the averages observed by the dilutions of the anthelmintics in comparisons within the groups. This may suggest that the fungus *A. musiformis* was not directly affected by the tested concentrations of abamectin, albendazole and levamisole. It is possible that this fungus has some resistance to the growth interferences that the anthelmintics could cause at the dosages tested in this trial.

The authors Wang et al 2021 tested fungi among the species of *Arthrobotrys* sp., and showed that from 10 drugs; including the anthelmintics fenbendazole, thiabendazole, ivermectin and levamisole, as well as the antifungals carbendazim, metalaxyl, difenoconazole pentachloronitrobenzene (PCNB), amphotericin B and ketoconazole; all had inhibitory effects on the fungi, except for levamisole and PCNB. Although the drugs are different, there are similarities between the pharmacological classes, and in this study, both albendazole and levamisole had an effect over the growth of *A. musiformis*.

It is worth noting that the species used in this study (*A. musiformis* isolate I144) was not used by Wang et al 2021, using the species *Arthrobotrys oligospora (isolate* 447), *Arthrobotrys superba (isolate* 435) and *Arthrobotrys* sp. (isolate PS011), which indicates a possible variation among species or isolates of the fungi due to genetic factors.

It is important to understand the efficiency of integrated actions, such as the use of nematophagous fungi, which have become increasingly important, and there is a need to understand the relationship between the available forms of verminosis control, such as anthelmintic drugs. The elimination of anthelmintic residues in animal feces is of great importance, since the fecal bolus is where the main action of nematophagous fungi takes place Vieira et al 2020.

Albendazole is absorbed to a much greater extent than other benzimidazoles and is excreted in the urine over a period of nine days (28% within the first 24 hours). Albendazole is metabolized primarily into its sulfoxide and sulfone Nicholas et al 1992. As demonstrated by

Navrátilová et al 2023 carried out a study with albendazole (ABZ) and found two main metabolites of ABZ, ABZ-sulfoxide (active anthelmintic) and ABZ-sulfone (inactive), which persisted in the soil (up to 25 cm from the feces) and in plants for three months after the end of the experimental period. According to Spinosa, Górniak and Bernardi 2017, the formation of sulfoxide and sulfone can increase the anthelmintic activity of these compounds, and their characteristic insolubility in water and most organic solvents allow them to remain active for longer periods of exposure. This may be associated with the effect of this anthelmintic found in this study, which had significant effects on the fungi studied. Its characteristics of persistence, insolubility and elimination via urine make it especially important to understand its inhibitory effect over fungi.

Abamectin, from the avermectin group, are lipophilic compounds that can be soluble in various organic solvents, and are mostly excreted, approximately 98%, in their original form through feces Spinosa, Górniak and Bernardi 2017. Junco et al 2021 described that the adverse effects of the concentrations at which macrocyclic lactones are eliminated in the feces of domestic animals show that the use of these compounds can have compromising effects on the different species that colonize the manure, thus causing disintegration and incorporation into the soil.

Furthermore, it is necessary to understand the action of chemical controllers, such as levamisole, which belongs to the imidazothiazole group. This drug has an immunomodulatory action Purzyc and Calkosinki 1998 and is widely used as an anthelmintic, but also has anti-tumors and immunomodulatory effects in rheumatic diseases and hypersensitivity reactions Scott et al 1996. It has a plasma half-life of between 6-8 hours and 90% of the dose is eliminated in the urine in up to 24 hours Spinosa, Górniak and Bernardi 2017. Compared to the other drugs (albendazole and abamectin), this drug was the one that showed the least inhibitory action against certain fungi, which may be associated with its residence time and its greater solubility in water, losing its effect more quickly.

The action of levamisole described by Braga et al 2007, in an *in vivo* study of experimental lesions of dermatophytosis caused by *Microsporum canis* in rabbits, which were histologically assessed, found that the use of levamisole numerically reduced the score of skin lesions on the animals.

The drugs of the benzimidazole class, such as febendazole Oliveira et al 2020 and mebendazole Joffe et al 2017 were studied by the above authors as potential antifungals in the treatment of infections by the fungus *Cryptococcus* sp. The antifungal action was observed with a significant impact on the reduction of fungal structures and their proliferation rate Oliveira et al 2020; Truong et al 2018. Similar to what was found in the present study, with the action of albendazole (benzimidazole) against fungal growth.

No studies were found on the use of albendazole, although bases belonging to the same pharmacological class were found to be significant in affecting the growth of the fungus *Cryptococcus* sp., which demonstrates the relevance of this study in recognising the action of these drugs in influencing fungal growth.

Overall, the results of this study are highly relevant, highlighting the importance of assessing the sensitivity of the growth and development of different fungal species in the presence of anthelmintics.

The context of this study involved the relationship between the effects of anthelmintics on fungi. Furthermore, the effects of anthelmintics varied over time, suggesting that the response of fungi can change over time due to their high capacity for adaptation Persson and Jansson 1997. It is important to consider the varying factors when determining the impact of drugs on fungi. This observation may suggest that these drugs have an influence and compromise their use in synergism with anthelmintics in the control of helminthiasis at the level of fungal growth.

The studies by Joffe et al 2017, Oliveira et al 2020 and Truong et al 2018 were aimed at the direct intervention of anthelmintics in the growth of fungi, in order to use them as *Microsporum canis* controllers. Furthermore, no *in vivo* studies were found with the macrocyclic lactones to which the abamectin analyzed in this study belongs, verifying its potential effect as an antifungal. *In vitro studies* such as that by Zegbi et al 2024 have been verified.

Variation in the response of the fungi was noted in previous studies, such as Araújo, Santos and Ferraz 1993, Wang et al 2021 and Zegbi et al 2024, and in the present study over the seven days of evaluation and between the different concentrations of anthelmintics. This variation can be attributed to the different physiological and biological characteristics of fungi, which influence their sensitivity to chemical agents and their high capacity for adaptation. In addition, the activities of the drugs, their pharmacodynamics and pharmacokinetics, their formulation, pharmaceutical presentation and the execution of the test carried out.

It is essential to analyze the effects observed and try to identify the combinations that have the greatest potential for efficacy in controlling helminthiases, in order to optimize the results in practice. It is worth emphasizing that low concentrations, such as DL1 (albendazole and abamectin 1 ppm and levamisole 2.23 ppm) and DL2 (albendazole and abamectin 10 ppm and levamisole 22.3 ppm), initially showed no effect, but with the passage of time and exposure, the components generated by the fungi and the *in vitro* microenvironment may make them more susceptible to the effects of anthelmintics at these concentrations.

Rare were the studies found that analyzed the use of anthelmintics and their effect on the growth of nematophagous fungi. The studies by Araújo, Santos and Ferraz 1993, which

inspired the present study, analyzed the effect of anthelmintics on nematophagous fungi in specific dilutions and without checking positive controls and water.

Vieira et al 2016, carried out a study of the susceptibility of fungi to anthelmintic drugs, however, they did not describe their results of the relationship between the use of anthelmintics and fungal growth, but rather the description of the methodology on the minimum inhibitory concentrations (MIC).

In addition, Wang et al 2021, obtained similar responses for *Duddingtonia flagrans* and for the genus *Arthrobotrys*, different results were observed to those of the present study and Zegbi et al 2024 found that *Duddingtonia flagrans* under the effect of the anthelmintics ivermectin, albendazole and fenbendazole had inhibition of fungal growth, being similar in the drugs albendazole, which in both showed inhibition of mycelial growth of the fungi, as well as levamisole.

To summarize, it was important to see that there was an interaction between nematophagous fungi and anthelmintics in all the studies, demonstrating that it is necessary to understand the complex events of this association, whether through synergism of action against the parasites, or the direct action of the drugs inhibiting the action of the fungi.

Control methods for worms in cattle are complex and adaptable. Although they can be efficient, their effectiveness varies according to needs, strategies and adaptations. To obtain the best control effect, it is essential to adjust these strategies.

According to this study, one suggested approach to the use of anthelmintics in combination with fungi would be strategic control. This involves using the controllers at specific times, in isolation – applying anthelmintics on one occasion and fungi on another – in order to minimize the deleterious effects of anthelmintics on the fungi. Finally, another possibility is selective control, already established for sheep Joffe et al 2017, which uses criteria (FAMACHA and fecal examination) to carry out control interventions in the best way for cattle.

Conclusion

The results of this study revealed a connection between anthelmintics and fungi growth, highlighting the complexity of the interactions between them. It was observed that the fungi *A. musiformis* suffered less influence of the drugs albendazole and levamisole; the fungi *P. chlamydosporia* suffered less influence of levamisole with concentrations between 100 ppm and 1000 ppm; and the fungi *D. flagrans* suffered less influence of the drug abamectin.

These findings highlight the importance of considering these interactions when developing integrated parasite control programs for sustainable cattle farming.

References

Alizadeh A, Amin M, Khezri M, Saberi Riseh R (1560) Compatibility of *Beauveria bassiana* (Bals.) Vuill. with several pesticides. Int J Agric Biol 8530:1–9

Alves do Carmo T, Oliveira Mena M, de Almeida Cipriano I, et al (2023) Biological control of gastrointestinal nematodes in horses fed with grass in association with nematophagus fungi Duddingtonia flagrans and Pochonia chlamydosporia. Biol Control 182:105219. https://doi.org/10.1016/j.biocontrol.2023.105219

Araújo J V., Santos MA, Ferraz S, Maia AS (1993) Antagonistic effect of predacious Arthrobotrys fungi on infective Haemonchus placei larvae. J Helminthol 67:136–138. https://doi.org/10.1017/S0022149X00013018

Araújo JV (2023) Advances in the control of the helminthosis in domestic animals. Pathogens 12:1107. https://doi.org/10.3390/pathogens12091107

Araujo JM, Araújo JV de, Braga FR, et al (2009) Activity of the nematophagous fungi *Pochonia chlamydosporia*, *Duddingtonia flagrans* and *Monacrosporium thaumasium* on egg capsules of *Dipylidium caninum*. Vet Parasitol 166:86–89. https://doi.org/https://doi.org/10.1016/j.vetpar.2009.08.003

Ayupe T de H (2020) *Arthrobotrys cladodes var macroides* E *Pochonia chlamydosporia* Como Controladores Biológicos De Nematóides Parasitos Gastrintestinais De Equinos. Universidade Federal de Viçosa

Braga FR, Araújo JV De, Oliva Carvalho R, et al (2008) Observação in vitro da ação dos fungos nematòfagos *Duddingtonia flagrans*, *Monacrosporium thaumasium* e *Pochonia chlamydosporia* sobre ovos de *Eurytrema coelomaticum*. Parasitol Latinoam 63:. https://doi.org/10.4067/S0717-77122008000100007

Braga FR, Ferraz CM, da Silva EN, de Araújo JV (2020) Efficiency of the Bioverm® (Duddingtonia flagrans) fungal formulation to control in vivo and in vitro of Haemonchus contortus and Strongyloides papillosus in sheep. 3 Biotech 10:62. https://doi.org/10.1007/s13205-019-2042-8

Braga LT, Nunes-Pinheiro DCS, Sousa JAV de, et al (2007) Efeito do levamisol e do extrato etanólico de folhas de momordica charantia sobre a dermatofitose experimental em coelhos. 8:285–296. https://doi.org/https://doi.org/10.5216/cab.v8i2.1353

Cadioli MC, Santiago DC, Hoshino AT, Homechin M (2007) Crescimento micelial e parasitismo de *Paecilomyces lilacinus* sobre ovos de *Meloidogyne paranaensis* em diferentes temperaturas"in vitro". Ciência e Agrotecnologia 31:305–311. https://doi.org/10.1590/S1413-70542007000200006

Dackman C, Jansson H-B, Nordbring-Hertz B (1992) Nematophagous Fungi and Their Activities in Soil. In: Soil. Biochem.

Delmilho G, Bohland E, Stephanie N, et al (2024) Evaluation of the supply of Duddingtonia flagrans for the control of gastrointestinal parasites in sheep. An Acad Bras Cienc 96:. https://doi.org/10.1590/00013765202420220940

Ferreira DF (2011) Sisvar: a computer statistical analysis system. Ciência e Agrotecnologia 35:1039–1042. https://doi.org/10.1590/S1413-70542011000600001

Fonseca J dos S, Ferreira VM, Freitas SG de, et al (2022) Efficacy of a fungal formulation with the nematophagous fungus *Pochonia chlamydosporia* in the biological control of bovine nematodiosis. Pathogens 11:695. https://doi.org/10.3390/pathogens11060695

Girotto MJ, Aquino LFB, Perez RB, et al (2008) O uso de fungos nematófagos no controle biológico de nematódeos parasitas: Revisão de literatura. Rev Científica Eletrônica Med Veterinária 6:1–7.

Joffe LS, Schneider R, Lopes W, et al (2017) The Anti-helminthic Compound Mebendazole Has Multiple Antifungal Effects against Cryptococcus neoformans. Front Microbiol 8:. https://doi.org/10.3389/fmicb.2017.00535

Junco M, Iglesias LE, Sagués MF, et al (2021) Effect of macrocyclic lactones on nontarget coprophilic organisms: a review. Parasitol Res 120:773–783. https://doi.org/10.1007/s00436-021-07064-4

Melo ME de, Fonseca J dos S, Guariento EF, et al (2023) Eficácia de formulação fúngica associando os fungos nematófagos *Pochonia chlamydosporia* e *Duddingtonia flagrans* no controle biológico das nematodioses de ovinos em Seropédica-RJ, Brasil.

Neves PMOJ, Hirose E, Tchujo PT, Moino JR A (2001) Compatibility of entomopathogenic fungi with neonicotinoid insecticides. Neotrop Entomol 30:263–268. https://doi.org/10.1590/S1519-566X2001000200009

Nicholas H. Booth E Lesilie E. Mcdonald (1992) Farmacologia e Terapêutica em Veterinária, 1st ed. Guanabara, Rio de Janeiro

Nvrátilová M, Vokřál I, Krátký J, et al (2023) Albendazole from ovine excrements in soil and plants under real agricultural conditions: Distribution, persistence, and effects. Chemosphere 324:138343. https://doi.org/10.1016/j.chemosphere.2023.138343

Oliveira GL, Sobrinho ACS, Benjamim CJR, et al (2023) Physical training improves physical activity levels but is associated with amplification of sedentary behavior in older women. Front Public Heal 11:. https://doi.org/10.3389/fpubh.2023.1180901

Oliveira HC, Joffe LS, Simon KS, et al (2020) Fenbendazole Controls In Vitro Growth, Virulence Potential, and Animal Infection in the Cryptococcus Model. Antimicrob Agents Chemother 64:. https://doi.org/10.1128/AAC.00286-20

Oliveira I de C, Vieira ÍS, Freitas SG, et al (2022) *Monacrosporium sinense* and *Pochonia chlamydosporia* for the biological control of bovine infective larvae in *Brachiaria brizantha* pasture. Biol Control 171:104923. https://doi.org/10.1016/j.biocontrol.2022.104923

Persson C, Jansson HB (1997) Colonization of soil by nematophagous fungi. 127

Powers KG, Wood IB, Eckert J, et al (1982) World association for the advancement of veterinary parasitology (W.A.A.V.P.) guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine and ovine). Vet Parasitol 10:265–284. https://doi.org/https://doi.org/10.1016/0304-4017(82)90078-4

Purzyc L, Całkosiński I (1998) Ecto-ATPase from rat lymphocytes--in vivo studies on the influence of levamisole. Pol J Pharmacol 50:239–51

Rex JH, Alexander BD, Anders D, et al (2008) Reference method for broth dilution antifungal susceptibily testing of filamentous fungi; Approved Standard – Second Editon, M38-12, vol. 28 nº 16, Second Edi. CSLI Document M38-A2, Wayne, Pennsylvania

Scott DW, Miller Junior WH, Griffin CE, Figueiredto C (1996) Muller & Kirk dermatologia de pequenos animais. In: Interlivros (ed) Doenças imunológicas da pele, 5th ed. Rio de Janeiro, pp 448–580

Spinosa H, Górniak S, Bernardi M (2017) Agentes Antiparasitários. In: Farmacologia Aplicada à Medicina Veterinária, 6th ed. Guanabara Koogan, Rio de Janeiro, pp 563–574

Truong M, Monahan LG, Carter DA, Charles IG (2018) Repurposing drugs to fast-track therapeutic agents for the treatment of cryptococcosis. PeerJ 6:e4761. https://doi.org/10.7717/peerj.4761

Vieira ÍS, Oliveira I de C, Freitas SG, et al (2020) *Arthrobotrys cladodes* and *Pochonia chlamydosporia* in the biological control of nematodiosis in extensive bovine production system. Parasitology 147:699–705. https://doi.org/DOI: 10.1017/S0031182020000098 (b)

Vieira ÍS, Oliveira I de C, Campos AK, Araújo JV (2020) Arthrobotrys cladodes and Pochonia chlamydosporia: Nematicidal effects of single and combined use after passage through cattle gastrointestinal tract. Exp Parasitol 218:108005. https://doi.org/https://doi.org/10.1016/j.exppara.2020.108005 (a)

Vieira JN, Maia Filho FS, Ferreira GF, et al (2016) In vitro susceptibility of nematophagous fungi to antiparasitic drugs: interactions and implications for biological control. Brazilian J Biol 77:476–479. https://doi.org/10.1590/1519-6984.15715

Zegbi S, Sagües F, Saumell C, et al (2024) Effect of Different Anthelmintic Drugs on the Development and Efficacy of Duddingtonia flagrans. Ruminants 4:10–21. https://doi.org/10.3390/ruminants4010002

Wang B, Zhang N, Gong P, et al (2021) In vitro assays on the susceptibility of four species of nematophagous fungi to anthelmintics and chemical fungicides/antifungal drug. Lett Appl Microbiol 73:124–131. https://doi.org/10.1111/lam.13462

Wood IB, Amaral NK, Bairden K, et al (1995) World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). Vet Parasitol 58:181–213. https://doi.org/10.1016/0304-4017(95)00806-2.

6. CONCLUSÕES GERAIS

A integração revelou que os fungos nematófagos possuem um potencial significativo tanto como agentes complementares aos anti-helmínticos químicos quanto como métodos autônomos de controle biológico de parasitos, para reduzir significativamente as infecções parasitárias em bovinos.

A compreensão das variáveis ambientais foi essencial para garantir e verificar que os fungos isolados ou em interação ambiental potencializaram a redução de larvas de *Haemonchus* sp. e *Cooperia* sp. na pastagem e a redução do OPG com o uso de *Duddingtonia flagrans* e *Pochonia chlamydosporia,* isoladamente e em associação*,* embora os fatores climáticos sejam determinantes e isso deve ser levado em conta, o uso de controle anti-helmíntico com os fungos, favorece a abordagem principalmente em protocolos estratégicos em épocas como abril, maio e julho, na qual demonstrou que *D. flagrans* e *P. chlamydosporia* apresentaram ação e sua associação como controle pode ser benéfica. Já a verificação em condições laboratoriais do crescimento fúngico em contato direto com anti-helmínticos amplamente utilizados na rotina de controle da bovinocultura, reforçou ainda mais a eficiência dos fungos nematófagos, mostrando que esses organismos são capazes de atuar em sinergismo sem sofrer alterações de crescimento sob influência dos anti-helmínticos, os fungos *Arthrobotrys musiformis* e *P. chlamydosporia* sofreram menor influência em seu crescimento *in vitro* sob ação dos fármacos, diferentemente de *D. flagrans* que apresentou maior efeito de inibição em seu crescimento. Os achados sugerem que a integração de fungos nematófagos em estratégias de controle de helmintos pode representar um grande avanço para controle de helmintoses, promovendo uma abordagem mais sustentável e eficiente. A combinação de métodos biológicos e químicos, com os efeitos significativos nas infecções parasitárias em animais observados pelos fungos e que é possível realizar e utilizar em sinergismo com os anti-helmínticos, pelo crescimento não ser interrompido com a sua presença, respaldada por um entendimento robusto dos mecanismos de ação e sinergias, pode ser a chave para um controle mais eficaz e duradouro.

ANEXO A

CERTIFICADO

A Comissão de Ética no Uso de Animais - CEUA/UFV certifica que o processo nº 37/2020, intitulado "**Ação *in vivo* de *Duddingtonia flagrans* comparada a associação de *D. flagrans* e *Pochonia chlamydosporia* como controle biológico de nematoides em bovinos"**, coordenado pelo professor Jackson Victor de Araújo do Departamento de Veterinária, está de acordo com a Legislação vigente (Lei Nº 11.794, de 08 de outubro de 2008), as Resoluções Normativas editadas pelo CONCEA/MCTI, a DBCA (Diretriz Brasileira de Prática para o Cuidado e a Utilização de Animais para Fins Científicos e Didáticos) e as Diretrizes da Prática de Eutanásia preconizadas pelo CONCEA/MCTI, portanto sendo aprovado por esta Comissão em 12/10/2020, com validade de 12 meses.

CERTIFICATE

The Ethic Committee in Animal Use/UFV certify that the process number 37/2020, named **"In vivo action of Duddingtonia flagrans compared to the association of D. flagrans and Pochonia chlamydosporia as biological control of nematodes in cattle"**, is in agreement with the a actual Brazilian legislation (Lei Nº 11.794, 2008, Normative Resolutions edited by CONCEA/MCTI, the DBCA (Brazilian Practice Guideline for the Care and Use of Animals for Scientific and the Guidelines of Practice the Euthanasia recommended by CONCEA/MCTI therefore being approved by the Committee on December 16, 2020 valid for 12 months.

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Comissão de Ética no Uso de Animais – CEUA/UFV