




Review

# Recent Advances in the Control of Helminths of Domestic Animals by Helminthophagous Fungi

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**Abstract:** This review describes the advances acquired and proven in the use of helminthophagous fungi in the control of gastrointestinal helminth parasites in domestic animals. Old and well-known premises about parasitic epidemiology and the factors that can interfere with the best performance of biological control are mentioned. Some of the most promising fungi are *Duddingtonia flagrans* from the predatory fungi group and *Pochonia chlamydosporia* and *Mucor circinelloides* from the ovicidal fungi group. These fungi produce resistance spores called chlamydospores. Bioverm<sup>®</sup> and BioWorma<sup>®</sup>, based on the fungus *D. flagrans*, are available as commercial. Biotechnological products such as nanoparticles and obtaining primary and secondary metabolites have already been obtained from these fungi. Because they have different mechanisms of action, ovicidal and predatory fungi, when used together, can present a complementary and synergistic action in the biological control of helminths. Therefore, future research in the search for new formulations, the association of fungi from different groups, extraction of new molecules, and nanoparticles of these fungi in the control of helminths in various domestic animals are desired.

**Keywords:** nematophagous fungi; predatory fungi; ovicidal fungi; *Duddingtonia flagrans*; *Pochonia chlamydosporia*; *Mucor circinelloides*



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## 1. Brief History of Helminthophagous Fungi

There are many studies from across the world demonstrating the successful implementation of helminthophagous fungi for the biological control of parasites in animal production systems. It should be emphasized, a priori, that the term “helminthophagous fungi” is the best one to use due to the ability and diversified action of these organisms against not only nematodes but also cestodes and trematodes. However, the term nematophagous fungus is still the most used in the scientific literature.

Helminthophagous fungi can be divided into five groups: nematode-trapping/predatorial, opportunistic or ovicidal, endoparasitic, toxin-producing, and producers of special attack devices. The fungi of the first and second groups produce modified hyphae called traps, with which, by a mechanical/enzymatic process, they bind and digest nematode larvae, eggs, cysts, and nematode females [1,2]. Thus, they are the ones that best act in the predation of animal parasites. Supplied orally, after passing through the gastrointestinal tract of animals, fungal structures such as conidia, mycelium, and chlamydospores germinate in the feces, forming a network of hyphae with the ability to capture and destroy infective forms of animal parasitic helminths [3].

Since 2014, several studies carried out on an experimental basis have proposed the efficacy of helminthophagous fungi from the predatorial group (*Duddingtonia* spp., *Arthrobotrys* spp. and *Monacrosporium* spp.) and from the ovicidal group (*Pochonia chlamydosporia* and *Mucor circinelloides*) against the decrease in the rates of recurrence of helminth infections in different parts of the world [4–10]. In these studies, successful experiments demonstrated the great versatility of helminthophagous fungi in the classic biological control of helminths.

In the last five years, commercial formulations containing *D. flagrans* started became commercially available. In Brazil (Bioverm<sup>®</sup>—AC001, GhenVet Saúde Animal, Paulínia, Brazil), Australia, and New Zealand (BioWorma<sup>®</sup>—NCIMB 30336, BioWorma, Sydney, Australia), these products are already used, with administration in animal feed [8,11–14].

## 2. Most Promising Helminthophagous Fungi

### 2.1. *Duddingtonia flagrans*

The fungus *D. flagrans* acts as the main predatory fungus of gastrointestinal parasitic nematode larvae. Relevant results were published after using Bioverm<sup>®</sup> (GhenVet Saúde Animal, Paulínia, Brazil), a commercial product based on *D. flagrans* (AC001). Bioverm<sup>®</sup> is licensed by the Brazilian Ministry of Agriculture, Livestock, and Supply, and can be used to combat nematodiosis in domestic animals [8].

*Duddingtonia flagrans* was classified as “Old friend of researchers”, a tribute to the great diversity of action that this fungus has [3]. Over the years, a series of important studies demonstrating the effectiveness of strain AC001 in the production of extracellular enzymes and crude enzymatic extract denoted its ability to be tested against the control of helminth gastrointestinal parasites [9,15,16].

For decades, the good production of chlamydospores (resistant structures) of this fungus has been studied [17] and, therefore, its commercial potential. However, recently, the production of silver nanoparticles (AgNP's) derived from this fungus had an effect on infective larvae of *Ancylostoma caninum*, the main helminth of dogs and cats [18]. This finding opened new avenues for the use of the fungus and, therefore, new products such as bioanthelmintics may, in the future, be another possibility for a more successful parasite control against the already installed problem of parasitic resistance.

### 2.2. *Pochonia chlamydosporia*

The fungus *P. chlamydosporia* (Goddard) Zare and Gams (syn. *Verticillium chlamydosporium*) parasitizes helminth eggs through specialized structures called appressoria, which allow the colonization of the egg surface and penetration by mechanical and enzymatic action [19,20]. This fungus also produces chlamydospores in abundance. Several studies have addressed the efficacy of *P. chlamydosporia* in the control of helminths in domestic animals such as *Oxyuris equi* [21], *Ancylostoma ceylanicum*, *Ascaris suum* [22], *Fasciola hepatica* [23,24], *Toxocara canis* [25], and in poultry helminths [15,26,27].

There are reports that *P. chlamydosporia*, in addition to ovicidal action, parasitises female phytonematodes of the genus *Meloidogyne*, which are colonized and completely digested [28,29]. Several secondary metabolites produced by this fungus have been described as promising to be applied as anthelmintics [30,31]. Intermediate hosts of trematodes, such as the mollusk *Pseudosuccinea columella*, also have their offspring affected by this fungus [32].

Because they have different mechanisms of action, ovicidal and predatory fungi, when used together, can present a complementary and synergistic action in the biological control of helminths [33]. Therefore, the application of this fungus is of substantial importance for the biological control of helminths in domestic animals.

### 2.3. *Mucor circinelloides*

Another species capable of adhering to the surface of the eggs of certain helminths, penetrating and feeding on their contents, is *Mucor circinelloides* [34]. It is a filamentous sapro-

phytic fungus with action against trematode eggs (*F. hepatica*, *Calicophoron daubneyi*) [35,36], ascarids (*Toxocara canis*, *Toxascaris leonina*, *A. suum*, *Bayliscascaris procyonis*) [37,38], and whipworms (*Trichuris* spp.) [39]. In several investigations it was shown that *M. cicinelloides* can be cultivated together with *D. flagrans*, with ovicidal and larvicidal activity very practical for the control of helminths whose infective stages are eggs or larvae that develop in the environment [40].

### 3. Advances in the Control of Gastrointestinal Nematodes in Pasture Animals

The internal parasites of ruminants is considered one of the main causes of important economic losses in cattle kept under pasture conditions, mainly in tropical and subtropical regions, due to the conditions of high temperature and humidity [41,42]. The absence of adequate anthelmintic control can lead to significant losses responsible for the decrease in food consumption, gastrointestinal disturbances, and compromised animal development, which can result in death in extreme cases [43,44]. In some regions, helminthiasis are the main cause of mortality in goats, sheep, cattle, and horses, especially young animals. The parasitic nematodes are present year-round in pastures [45–47].

The anthelmintic resistance of gastrointestinal nematodes (GIN) has become an emerging problem worldwide [48–53]. Often, anthelmintics are administered without technical criteria for drug selection, empirically and indiscriminately, with additional implications for the efficacy of the products, causing the emergence and spread of anthelmintic resistance [54,55].

In tropical countries, the genera *Haemonchus*, *Cooperia*, *Trichostrongylus*, and *Oesophagostomum* are the most prevalent GIN in domestic ruminants, causing problems to animal health [55–58]. In horses, small strongyls (cyathostomins) and large strongyls (*Strongylus* spp.) are the most prevalent [47,59–61].

The free or pre-parasitic life stage of the main GIN of ruminants and horses is directly influenced by climatic variations in temperature, humidity, and rainfall, and the climate should be a major factor in the search for integrated measures for parasite control. The infective stages (L3) of the GIN have a greater rate of survival at the beginning of rainfall periods. However, under conditions of low rainfall associated with relatively mild temperatures, L3 may survive in the feces for extended periods, representing a source of contamination of pastures [41].

It is extremely important to recognize the problems caused by GIN throughout the world, highlighting that parasite control must, above all, start with knowledge about population dynamics and the epidemiology of GIN, their characteristics and main species present in the most diverse types of ruminants and equines productions worldwide. The knowledge of the degree of contamination of pastures by infective strongylide larvae is very useful for epidemiological purposes. It can determine the risk of infection in animals and provide data for the establishment of integrated control programs [45].

In the environment, helminthophagous fungi can survive as saprophytic agents, feeding on environmental organic matter, or as parasites, feeding on a wide variety of free-living helminths [62,63]. Factors such as temperature, humidity, and oxygen, which determine a microclimate favorable to the development of helminth eggs and larvae, also enable fungal survival, making possible the predation of parasites [64–66].

It has been proven that after oral administration of *D. flagrans* (AC001-Bioverm<sup>®</sup>, GhenVet Saúde Animal, Paulínia, Brazil), at a dosage of 1 g/10 kg of body weight ( $10^5$  chlamydospores/g), there was fungal survival after passage through the gastrointestinal tract of sheep and cattle, with efficient larval predation in vitro [8,14]. Under field conditions, the daily administration of Bioverm<sup>®</sup> for six months demonstrated a significant parasitic reduction in cattle and horses in Brazil [12,13]. The use of biological control by helminthophagous fungi provides an option to reduce the rates of contamination by larvae and, consequently, less reinfection for the animals, enabling them to develop natural immunity against the helminths. In addition, animals that receive helminthophagous fungi have higher weight gain rates, higher globular volume percentages, and lower EPG values

when compared to those that do not receive the fungi [5,67–70]. In grazing horses that received *D. flagrans* chlamydospores daily, strongylide counts below 200 EPG were obtained for 16 months, also verifying that they recovered normal blood values of erythrocytes, hemoglobin, and hematocrit [71]. With daily administration of a mixture of *M. circinelloides* and *D. flagrans* chlamydospores, a grazing dairy herd was not reinfected by *C. daubneyi* for a period of two years, and the strongylide count in feces was less than 110 EPG. No adverse effects were detected in any animal [72].

#### 4. Advances and Perspectives for the Control of Geohelminths

The nematodes *A. caninum*, *T. Canis*, and *T. cati* are potentially zoonotic parasites of dogs and cats in various parts of the world [73–75]. As part of the environmental cycle, these parasites manage to cause infections via soil/environment, giving them the name of geohelminths [76].

The use of anthelmintic drugs is the main form of control used to helminth infections in domestic animals, but cases of therapeutic failure have also been observed, resulting in reports of anthelmintic resistance [77–79]. It is noteworthy that the high environmental resistance of infective *A. caninum* larvae, *T. Canis*, and *T. cati* eggs gives them a greater ability to survive. However, by the use of helminthophagous fungi *P. chlamydosporia*, the environment can be used as an alternative route for their control [80,81].

In places where there is a high population of stray dogs and cats, the presence of zoonotic parasitic forms in the soil of public squares is a sanitary and environmental problem [82,83]. Children are the main affected, as they have the habit of playing on the ground [84]. The soils of public environments can be considered maintenance areas for parasites with zoonotic potential, with difficult environmental control, since restricting the access of animals to these environments is impractical. Common disinfectants such as 2% sodium hypochlorite (bleach), have highly effective in environmental sanitation, with the destruction of geohelminths in impermeable soils and surfaces. Nonetheless, common disinfectants have limitations of effectiveness on other surfaces, such as sandy soils and lawns [85,86]. Research with the purpose of promoting integrative control measures, through the association of chemical and biological control, has been developed and demonstrated satisfactory results [3,9,40,80,87,88]. Research should be encouraged to evaluate the associated use of helminthophagous fungi and common disinfectants, enabling their associated use in doses that do not cause fungal inhibition, increasing the use of these fungi.

#### 5. Biotechnological Advances in the Use of Helminthophagous Fungi

The understanding of biotechnological advances with the use of helminthophagous fungi can be divided into two main points: predatory action against infective larvae and helminth eggs; and the production of primary and secondary metabolites [89]. In this sense, the predatory activity of helminthophagous fungi and its mechanism of action through traps and/or adhesive nets has long been recognized [19,90–93]. For this reason, the use of fungal mixtures with complementary activity (ovicide and larvicide) is of great importance. Furthermore, this mixture of fungi resists the process of manufacturing nutritional pellets and, therefore, represents a very useful formulation for the control of different helminths that affect domestic animals in grazing and wild animals in captivity [40,94].

The production of extracellular enzymes is important for understanding the mechanical and enzymatic process carried out by helminthophagous fungi, especially those classified as ovicides [89]. Through biochemical and molecular studies, it was found that there was a great possibility that these fungi produce extracellular enzymes with direct use against nematode larvae and eggs [95].

The enzymatic activity of nematophagous fungi has motivated great interest in studies in different countries. For example, in China, a recombinant protein from the species *Arthrobotrys oligospora*, one of the most widely studied nematode-trapping fungi, has been demonstrated to have a high chitinase activity and is able to degrade chitin from both

infective stages and the egg-shell of the following helminth species *Strongylus equinus*, *Caenorhabditis elegans*, and *Haemonchus contortus* and also the egg-shell of the trematodes *F. hepatica* and *Dicrocoelium chinensis* [96].

Thus, research has advanced, and, recently, a new possibility of using these fungi in the control of nematodes was discovered through nanoparticles (NP's) biosynthesized from fungal filtrate [97]. It has been proven that nanoparticles biosynthesized by *D. flagrans*, were able to cause the destruction of infective larvae of *A. caninum* [18].

The biosynthesis of these nanoparticles by filamentous fungi has attracted considerable interest for the use of these microorganisms in the production of NP's, due to their ability to grow on low-cost, nutrient-poor substrates and to produce secondary metabolites. In addition, fungal mycelium can withstand flow pressure, agitation, and other laboratory conditions important for large-scale production. Still, in biological synthesis using fungi, the biosynthesis of NP's occurs extracellularly. That is, the reduction occurs outside the cell, eliminating the need for extra steps to coat the NP's in post-production [98].

Helminthophagous fungi can also produce ecological silver nanoparticles (AgNP's) [18,97,99]. They convert toxic metal ions into non-toxic nanoparticles through their catalytic effect [100]. The destruction of L3 of equine cyatostomine nematodes by AgNP's—*D. flagrans*, with nematicidal effect in 24 h, was a great advance for a new use of these fungus [9].

In the fungus *P. chlamidosporia*, the secondary metabolite ketamine showed excellent efficacy against *A. ceylanicum* and *A. suum* and with similar efficacy to the anthelmintic albendazole [31]. Therefore, future research in the search for new formulations, association of fungi from different groups, extraction of new molecules and nanoparticles are desired.

Regarding the study of nematocidal activity of nematophagous fungi metabolites, most research studies have been focused on assessing the nematocidal activity of these molecules against nematodes of importance in agriculture, and there is only scarce information about nematophagous fungi products against parasitic nematodes of impact in the area of animal production. Thus, this is an opportunity to explore this wide and promising field of research to find other biotechnological uses and applications of nematophagous fungi to benefit farmers all over the world.

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