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Advantageous Fungi against Parasites Transmitted through Soil

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Abstract

Although many fungal specimens are responsible for human and/or animal infection, other species are advantageous for preventing the infection by soil-transmitted zoonotic parasites. Infection occurs by the accidental ingestion of parasitic stages (cysts, oocysts, eggs, and larvae), their active penetration through the skin or through direct contact. Numerous species of helminths develop an external phase in the soil where the infective stages are attained, thus mammals become infected when grazing, drinking, or accidentally. Ectoparasites as ticks perform also in the soil the phase from egg to larva. Different soil saprophytic fungi that turn into predatory agents when parasitic stages are near have been isolated and described. These species are capable of destroying the pathogens or irreversibly decreasing their viability, providing thus a very interesting and sustainable tool to reduce environmental contamination by pathogenic agents. In the last year, a profound knowledge on the most appropriate fungal species, together with the proper way to disseminate them, has been acquired.

Keywords: *Mucor circinelloides*, *Duddingtonia flagrans*, parasiticide, soil, STHs, zoonoses

1. Introduction

1.1 Organisms in soil

The definition of soil according to the sciences of the earth and life points to the external part of the earth's crust, which is biologically active and tends to develop on the surface of the rocks emerged by the influence of weather and living beings. It is also frequent that this concept includes a complex set of physical, chemical, and biological elements that make up the natural substrate in which life develops on the

surface of the continents. The soil is the habitat of a specific biota of microorganisms (bacteria and fungi), plants, and small animals that constitute the edaphon.

In recent decades, there has been an increasing concern for soil biodiversity, on the basis that the interactions between microorganisms, animals, and plants provide an undoubted benefit to the well-being of mammalian species, including man [1]. This biodiversity conditions both the possibilities of feeding these species, oxygenation, as well as the control of the risk of certain diseases. For these reasons, it is not difficult to understand that soil biodiversity is directly affected by global changes caused by man, especially those related to land use, urbanization, agriculture, deforestation, and desertification, which leads to the logical conclusion that the careful and sustainable use of soils would guarantee their benefits.

Different studies have indicated that exposure to soil microorganisms decreases the prevalence of allergic diseases [2]; taking into account the predictions that around the year 2050 two-thirds of the world population will reside in cities, the stimulation of the immune system by soil organisms will be reduced, and therefore allergy cases will increase.

Other researches highlight the increase in the appearance of bacterial species resistant to most known antibiotics, and the same happens with some parasites, such as helminths. The use of remedies found in the soil, such as certain types of fungi, has not yet come to be considered as a solution to the aforementioned problems. It is interesting to know that some bacteria capable of synthesizing effective antibiotics against *Mycobacterium tuberculosis* have been isolated in the soil [3]. It should also be noted the production of molecules with parasiticide action from fungi [4]. Special mention should be made of the use of some fungal species in the control of certain endoparasites that, once in the soil, complete a series of phases until they reach the infective stage [5]. In recent years, very important achievements have been made in the large-scale production of saprophytic fungal spores that are found in the soil, such as *Mucor circinelloides* or *Duddingtonia flagrans*, filamentous species that are in contact with eggs or larvae of some parasites, respectively. They have the capacity to destroy them or limit their viability [6, 7]. In this way, it is possible to reduce the risk of infection in people, and also in animals that are in pasture.

1.2 Pathogenic organisms transmitted through the soil

Pathogenic organisms belong mainly to five main groups, viruses, bacteria, fungi, and parasites (protozoa, helminths, and ectoparasites) [8]. From an academic and disease control approach, the importance of soil lies in the fact that a significant number of pathogens are found in this habitat, and sometimes they are accidentally ingested by animals and people, causing important disorders. There are some organisms that do not require ingestion, being able to spread their pathogenicity through bites or penetrating the skin.

Table 1 summarizes different examples of pathogens present in the soil. It is important to note that most soil organisms do not constitute a health risk, and pathogenic species represent only a minority. Nor should we forget that some species are opportunistic (*Pseudomonas* and *Enterobacter*) and can cause alterations in mammals, although in the soil they are actually antagonists of root pathogens of some plant species, or can act as growth promoters of some plants and even as decomposers of organic matter [9]. Other pathogens need to develop part of their cycle in the soil, to complete their evolution until the infective phase. These are organisms that can survive in the soil for long periods of time, and include spores, eggs, or even larvae. These are obligate pathogens that temporarily reside in the soil, and that are transmitted to mammals by direct contact, by vectors, or through accidental ingestion [10]. For these reasons, it is necessary to know the ecology of

Bacteria	<i>Bacillus anthracis</i>	<i>Agrobacterium tumefaciens</i>
	<i>Listeria monocytogenes</i>	<i>Escherichia coli</i>
	<i>Salmonella</i> spp.	<i>Clostridium</i> spp.
Fungi	<i>Aspergillus</i> spp.	<i>Histoplasma capsulatum</i>
	<i>Coccidioides immitis</i>	
Protozoa	<i>Naegleria fowleri</i>	<i>Toxoplasma gondii</i>
Helminths	<i>Ascaris</i> spp.	<i>Taenia</i> spp.
	<i>Ancylostoma</i> spp.	<i>Strongylus</i> spp.
Ectoparasites	<i>Pulex irritans</i>	<i>Ixodes</i> spp.

Table 1.
Numerous pathogens can be found in the soil.

the interactions between the soil and the various organisms to determine why some are prevalent and persist under certain conditions.

The concept of *Soil Borne Human Diseases* offers a very accurate introduction about the role that soil can play in the transmission of certain diseases [11]. However, it is obvious that this idea is a bit limited, since not only the human species will experience the risk of contracting diseases from this habitat. From an etiological point of view, *pathogenic organisms* are defined as those whose habitat is the soil, and *pathogens transmitted by the soil* as organisms that can survive for long periods of time in the soil, and need to do so to infect the host and continue their biological cycle, but they are not part of the soil [12]. Some of the most frequent endoparasites affecting people and animals, such as roundworms, cestodes, or strongyles, belong to this group, and they are characterized by undergoing a series of changes in the soil to the infecting stage. Part of the biological cycle of some ectoparasites such as fleas or ticks occurs in the soil also. This underlines the importance of soil as an adequate medium to certain parasites can survive and develop to infective stages, pending of proper hosts ingest them (flatworms, roundworms, whipworms), contact with soil (hookworms) or walk near (ectoparasites). Regardless of their origin (animal/human), control of parasites affecting mammals requires some action on the stages in the group, since parasiticide therapy acts on the parasites living and affecting them only; thus, the risk of reinfection is elevated, even though successful treatments are applied.

1.3 Mammal parasites developing in the soil

Soil provides a suitable habitat to different organisms as plants can grow and develop, serving as food for the survival of many living creatures (insects and micro-mammals). This environment enables mammals as herbivores to graze and carnivores to find their feeding.

Most known parasites associated to soil are defined as *soil-transmitted helminths* (STHs), which involve well-known species belonging to flatworms, tapeworms, or roundworms. Helminths can develop a direct cycle in the soil, but an intermediate host is required for some species, and paratenic hosts participate in the transmission of several infections. On the basis of the zoonotic role of different parasites developing in the soil, it is necessary to know the external phase of their life cycle.

Transmission of STHs involves that eggs are passed in the feces of infected individuals. Once in the soil, flatworms (trematodes and cestodes) need to complete several stages inside an intermediate host, to attain the infective stage. *Fasciola hepatica* and *Schistosoma mansoni* (flatworms) are related to humid environments where a number of aquatic or amphibious snails take part. After some stages are completed and exit-off the snails, the infective stages known as metacercariae mature in herbage or water, and infection occurs by the ingestion of herbage or water contaminated [13].

Roundworms (*Ascarids*) represent the most spread nematodes around the world. Although these are host species-specific pathogens, humans can be involved as paratenic hosts for many of them such as roundworms infecting domestic animals (*Toxocara canis*, *Ascaris suum*) or wild species (*Baylisascaris procyonis* and *Toxascaris leonina*). Infection occurs by the accidental ingestion of larvated eggs (containing a second-stage larva inside) (**Figure 1**).

Whipworms (*Trichuris* spp.) have a similar cycle to roundworms. Transmission occurs by the oral ingestion of eggs holding a first larva.

In the case of *Ancylostoma*, nematodes (hookworms), embryonated eggs are passed in the feces and once in the soil, the first-stage larva (L3) emerges and molts to a second-stage larva and then to a third-stage larva, the infective stage. Infection can occur either by oral ingestion of L3 or through the skin [14].

It is well recognized that ticks need to suck blood from mammals for surviving, but sometimes it is forgotten that these ectoparasites develop part of their life cycle in the soil also. Gravid adult females drop off the final host to the ground to lay eggs. Under appropriate conditions, the egg hatches into a larva, which waits for an appropriate mammal to bite for feeding and then transform into nymph.

Appropriate conditions (moisture and temperature) must concur in the soil to improve the development of parasites to their infective stages. Nevertheless, evolution of parasites can be delayed until unfavorable circumstances appear, especially low temperatures. Some of them such as roundworms and whipworms are able to survive viable for long periods, even under temperatures below zero [15]. This

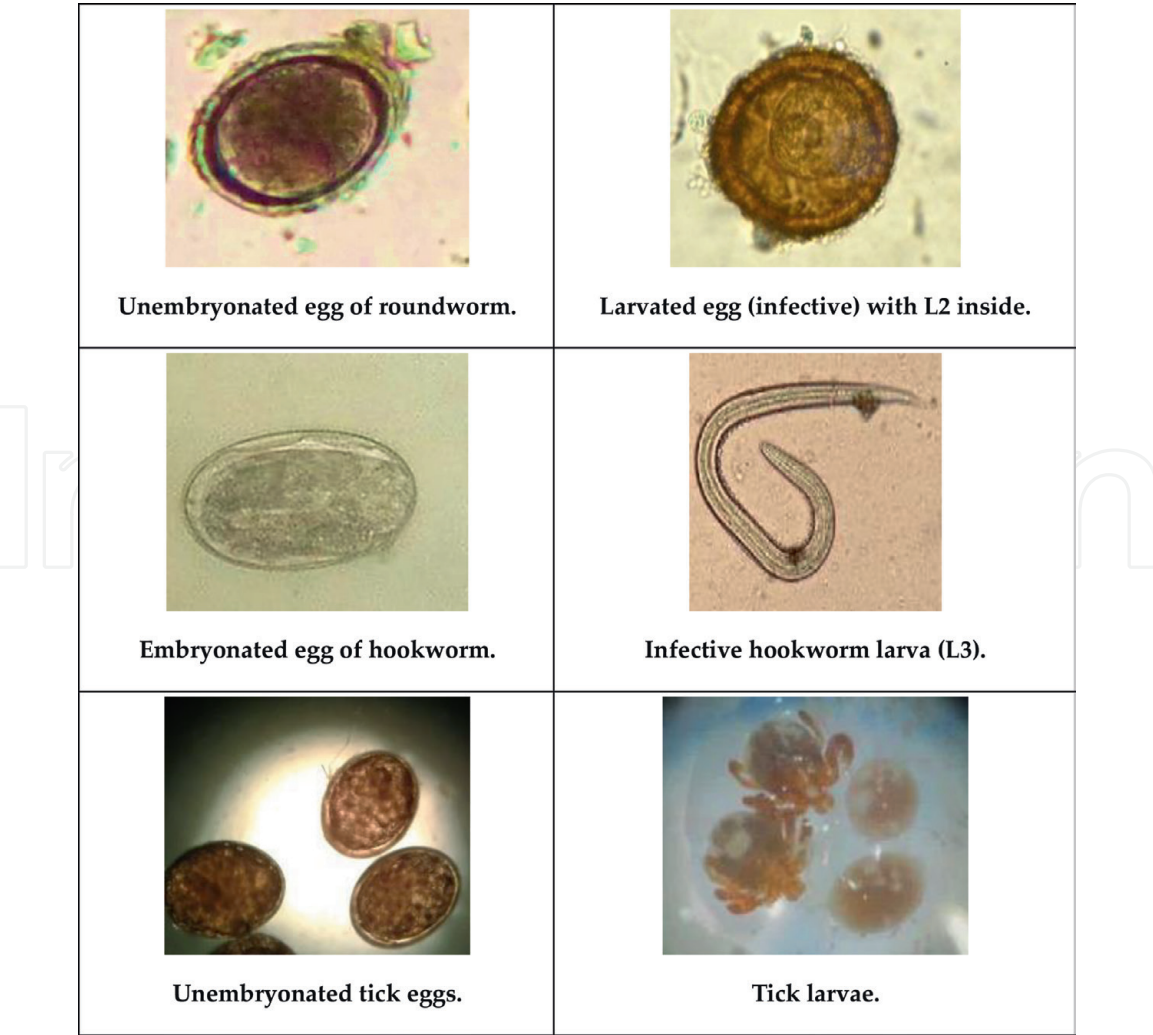


Figure 1.
Numerous parasitic stages can be found in the soil (COPAR archive).

resistance is conferred by their eggshells, composed of at least four layers, uterine (mucopolysaccharides), vitelin, chitinous, and lipidic (inner). Eggs of ticks can also survive in the environment unless the solar light falls directly on them.

Larval stages (first, second, or third) from nematodes exhibit a certain degree of resistance, and it has been reported they can subsist under snowy areas [16]. Dry soils in spite of very humid areas are preferred by immature hookworms [5], like sandy places. This explains the cutaneous infection of people enjoying outdoor activities on beaches, parks, etc. from touristic areas.

1.4 Importance of infection by parasites from the soil

Human STHs are frequent in Asia, Africa, and South America, being absent in Western Europe and developed countries. Nevertheless, these diseases have reemerged due to immigration, travel, and business. Also in recent years, populations of ticks are increasing in urban areas, as well as orchards, parks, and gardens [17].

There are four main STHs affecting humans, *Toxocara canis* (round-worm), *Ancylostoma duodenale* and *Necator americanus* (hookworm), and *Trichuris trichiura* (whipworm). Between 1.5 and 2 billion people, it is believed that they are probably infected worldwide [18]. The presence of these parasites is associated to low standards of hygiene, poverty, and malnutrition because infection takes place by the accidental ingestion of eggs or through cutaneous contact with larvae of hookworms. It is necessary the exposure to feces of pets, mainly dogs. As advised by the WHO (World Health Organization), periodic administration of albendazole and mebendazole is helpful to reduce the incidence of these parasites. Deworming is the most applied measure against STHs, and extension of treatment (increment of frequency) looks like a valuable solution, although there is a potential emergence of drug resistance as observed in veterinary medicine [19]. By considering that infections originate from fecal contamination of the environment, mammals can become reinfected frequently after parasiticide treatment is administered. Consequently, actions on the environment are required to reduce the exposure to infective stages, mainly consisting of the use of latrines, together with hygienic behaviors.

Dogs are the definite hosts for *T. canis* and *N. americanus*, thus another question to address concerns the possibility of humans and animals sharing infections by parasites, the so-called parasitic zoonoses. As explained previously, transmission occurs in the same way, but the presence of infected animals becomes essential for human infection. In this case, control appears more difficult, due to the impossibility to ensure that pets receive an appropriate deworming therapy. The problem aggravates when considering that wild/uncontrolled animals can live near persons, because there is no way to perform control of their parasites by the administration of antiparasitic drugs. In some countries, it is not rare to observe feces of stray dogs or cats, foxes or raccoons, in private gardens, public parks, or even beaches. As stated above, humans might become infected by roundworms or hookworms, and despite infection, it is not completed, serious damage could be provoked attributable to the erratic migration of immature stages across the organism [20]. At this point, it seems necessary to remember that second-stage larvae of *Toxocara canis* (dog), *T. cati* (cat), *Ascaris suum* (pig), or *Baylisascaris procyonis* (raccoon) can cause a visceral larva migrans syndrome after these larvae are released at the gut level. Infection by *T. canis* can be responsible for an ocular larva migrans [21], while *B. procyonis* is associated to a devastating neurological syndrome, with children being the riskiest group due to their tendency to play with ground, or take and leave sand in the mouth [22]. The possibility of human infection through the exposure to eggs of roundworms on the coat of dogs has also

been considered [23], which remarks the importance of these parasites are easily transmitted to their owners.

2. Beneficial soil fungi

2.1 Antagonists of helminths

By considering that a great number of pathogens develop in the soil, one interesting question refers to why mammals did not infect more frequently, or why low to moderate infections are usually detected. Infection depends on the density of pathogens and risky situations such as accidental ingestion or active passage through the skin (helminths) or walking by places with vegetation (ectoparasites). Then, it could be expected that exposure to natural environments might represent a great hazard, thus enjoying natural habitats should be avoided (or even forbidden).

As mentioned previously, a great number of fungal species can be found in the soil, together with many other organisms such as viruses, bacteria, earthworms, insects, etc. Some of these species are saprophytic and feed on organic matter, but in the presence of parasitic stages such as eggs or larvae, they shift to predatory agents. Hyphae develop and the mycelium grows toward the parasites in an attempt to take certain nutrients, nitrogen and carbon mainly [24]. Other fungal species feed on different species of fungi, as succeeds with some mites.

It has been demonstrated that certain soil saprophytic fungi such as *Duddingtonia flagrans* are able to adapt to the numbers of larvae of nematodes developing from eggs shed in feces of infected grazing horses [25]. In the absence of nutrients, fungi can remain as resting stages (spores). It should be emphasized that different organisms interact simultaneously on the ground, thus soil fungi do not persist for long periods (4 months) and need to be replaced by new structures such as spores, mycelium, etc. [26]. This must be taken into account when soil fungi are going to be used under biological control strategies. Other interesting finding consists of the absence of activity on nonparasitic organisms (**Figure 2**).

Based on experiments with plants, traditionally the fungal antagonists of parasites comprise nematode-trapping species (larvicidal), predacious agents, endoparasitic fungi, and egg parasitic fungi (ovicidal) [27]. In the last decades, this classification applies also for defining the activity of soil fungi against parasites affecting mammals (**Table 2**).

In natural conditions, when the environment does not result altered by humans, soil albeits not only fungi but other microorganisms as viruses, bacteria, earthworms, insects... A number of filamentous fungi feed on organic detritus, certain

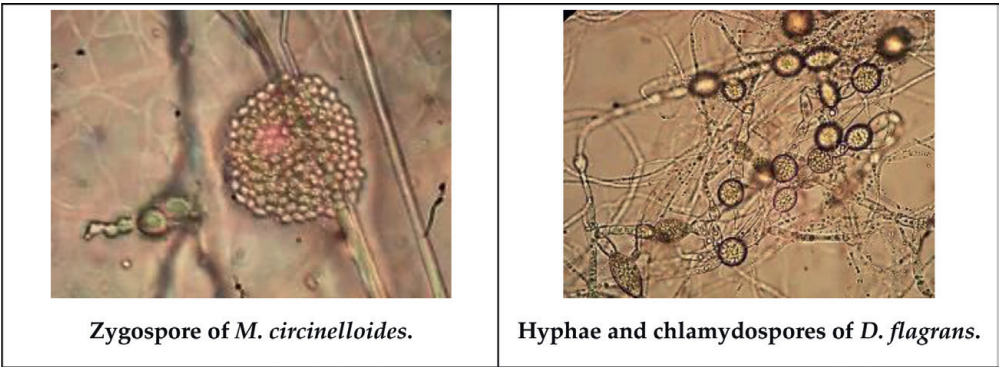


Figure 2.
Filamentous fungi develop hyphal nets in the soil, and reproduce by spores (COPAR archive).

Effect	Species		Action against
Ovicidal	<i>Pochonia chlamydosporia</i>	<i>Mucor circinelloides</i>	Flatworms
	<i>Purpureocillium lilacinus</i>	<i>Verticillium chlamydosporium</i>	Roundworms (ascarids)
	<i>Trichoderma</i> spp.	<i>Gliocladium</i> spp.	Whipworms
Larvicidal	<i>Duddingtonia flagrans</i>	<i>Monacrosporium</i> spp.	Hookworms
	<i>Arthrobotrys</i> spp.		Roundworms (strongyles)

Table 2.
Filamentous soil fungi antagonists of parasites in the soil.

coprophagous beetles participate in enriching the ground by decomposing organic matter as manure, some mites feed on fungi, and several fungi do it also. This means that an equilibrium situation takes place, where organisms are controlled mutually, and explains also why low risk of infection is usually observed. When agricultural procedures affecting the surface of the ground are performed, this habitat is transformed, and beneficial organisms drop or disappear. As a consequence, the density of pathogens increases, accordingly the risk of exposure among mammals increases and they can become infected.

Several investigations pointed the efficacy provided by some fungi to limit the viability of eggs of roundworms [28]. As drawn in **Figures 3** and **4**, the addition of spores of *M. circinelloides* to the feces of dogs infected by *T. canis* decreased their viability by half after a period of 30 days [29]. When the spores were sprayed onto feces of raccoons parasitized by *B. procyonis*, egg viability reduced by two-thirds also, in agreement with previous experiments [30].

A notable efficacy has been reported against larvae of hookworms by using trapping-nematode fungi such as *D. flagrans*. A 57–73.2% reduction of the numbers of the third-stage larvae of *Ancylostoma* spp. has been obtained, and the counts of larvae decreased by 24.5–63% when exposed to chlamydospores of *D. flagrans* [31, 32].

By taking into account that the aforementioned parasites are STHs, the use of ovicidal and larvicidal fungi could be strongly helpful to limit the development of parasites to infective stages in the soil. One interesting question refers to the proper way to spread the fungi to ensure their contact with the parasites. Because the eggs of parasites are shed by feces, the most useful procedure looks to try that fungi

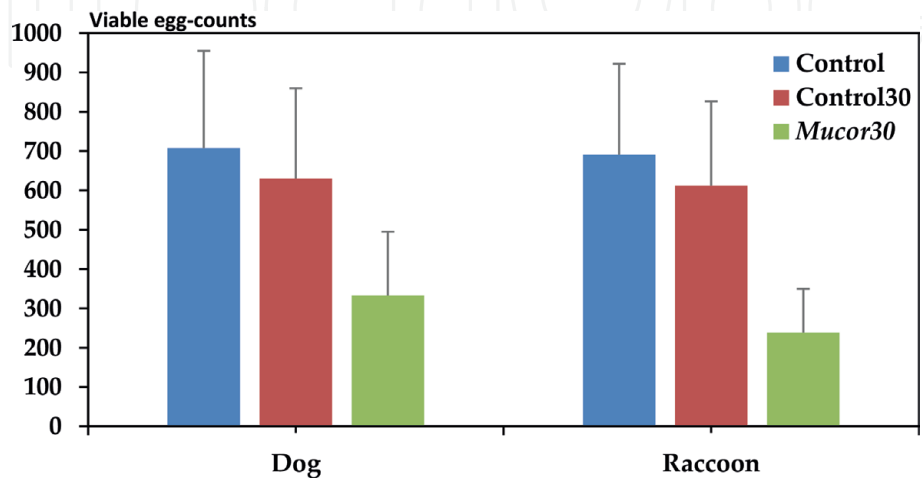


Figure 3.
*Viability of eggs of *Toxocara canis* (left) and *Baylisascaris procyonis* (right) after 30 days of exposure to spores of *Mucor circinelloides* (Mucor30) or distilled water (Control 30). Points mean average values and bars the SD [29].*

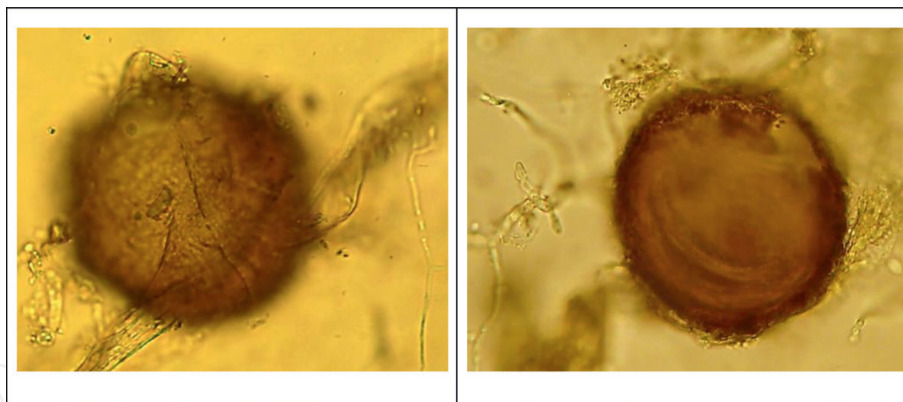


Figure 4.

The soil filamentous fungus *M. circinelloides* is able to attach to the eggshells of roundworms such as *T. canis*, colonize, penetrate, and absorb the inner content (COPAR archive).

are in the feces at the same time, and for this purpose, oral administration could be appropriate. Several investigations demonstrated that the spores of *Pochonia chlamydosporia*, *Mucor circinelloides*, and *Duddingtonia flagrans* can survive the passage through the gastrointestinal tract of different animal species, and retained their antagonistic activity [6, 33, 34]. Later, several assays were performed by adding spores or mycelium of *Pochonia chlamydosporia* or *Duddingtonia flagrans* during the handmade elaboration of nutritional pellets [35–37]. More recently, the capability of fungal spores to resist the industrial fabrication of pelleted feed has been demonstrated [38, 39]. The usefulness of pellets containing spores of *M. circinelloides* and *D. flagrans* has been tested on grazing horses, and highly successful results were obtained. Through this strategy, it was possible to reduce the frequency of deworming from 4 years to 1–1.5 years [7, 40]. This approach has also been assayed on wild captive equids maintained in a zoological park, and as a result the administration of anthelmintics was significantly lessened [41], supporting the results previously collected by administering the spores as a premixed feed [6].

2.2 Entomopathological agents

It has been explained that ectoparasites develop part of their cycle in the soil. After mating on the host, gravid female ticks engorge completely and drop to the ground, where thousands of eggs are laid mainly in places protected from sun and desiccation, with vegetation. Later than a variable period, depending on temperature and humidity, eggs hatch and larvae exit off, addressing to plants, pending of a host to attach and suck blood for molting into nymphae. *Beauveria bassiana* and especially *Metarhizium anisopliae* are the most investigated entomopathogenic fungi capable of infecting and damaging ticks [42, 43]. Trials consisted of the topical administration of oil solutions, targeted against immature or adult stages [44]. The aim is to reduce the indiscriminate use of chemical acaricides, for avoiding contamination of food and environment, as well as the appearance of chemical resistance among tick populations [45].

There is little information available concerning the possible effect of fungi on tick eggs in the soil. **Figure 5** summarizes the results collected after the exposure of eggs of *Rhipicephalus boophilus* to spores of *M. circinelloides*. The fungal activity was estimated by measuring the percentage of egg viability, and the hatching percentage, i.e., the percentage of larvae hatched after 15 days. Fungal growth started on the eggshells 4 days after exposure, and by 6 days, hyphae penetrated inside.

Viability of ticks' eggs decreased to 80% in the controls-untreated eggs, and to 38% in those exposed to the filamentous fungus. The hatching percentage was 45% in the controls, by 15% in the *Mucor*-treated eggs.

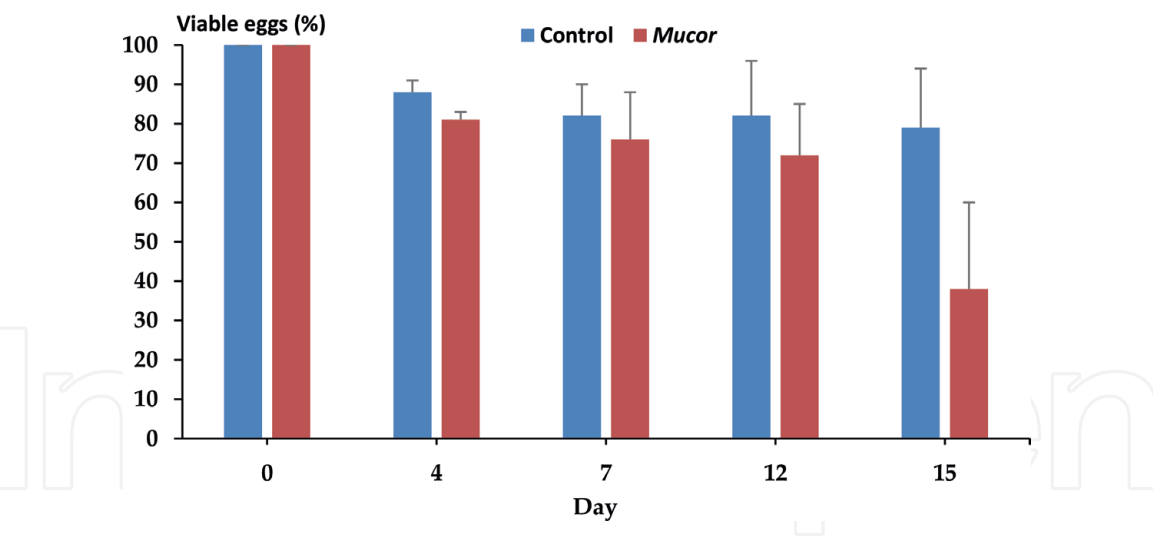


Figure 5. Viability of eggs of the tick *R. boophilus* exposed to spores of *M. circinelloides* (*Mucor*) or distilled water (Control). Points mean average values and bars the SD (COPAR archive).

Four phases have been described during the activity that the ovicidal fungus *Verticillium chlamydosporium* perform on eggs of helminths, i.e., contact, attachment, penetration, and deliberation [46]. The fungus *M. circinelloides* develops a similar activity on both the eggs of helminths and ticks (**Figure 6**). When the spores contact with the parasites, hyphae grow toward the eggshell and colonize it. Those hyphae facing the eggshell in perpendicular are able to penetrate inside. This is possible due to the involvement of the *appressorium*, a pressing organ consisting of a flattened and thickened hypha, which is provided of a *haustorium*, a specialized branch which penetrates the tissues of the host and absorbs nutrients and water [47]. This mechanism enables the fungus to take all the inner content of the egg, without losing anything. Once completed, hyphae exit off and colonize other egg (deliberation).

In view of the mentioned results, certain soil fungi seem very promising agents for limiting the viability and evolution of tick eggs in the soil, contributing to decrease the risk of infestation. One possible approach could rely on preparing aqueous solutions containing the fungal spores, and spreading by using airless

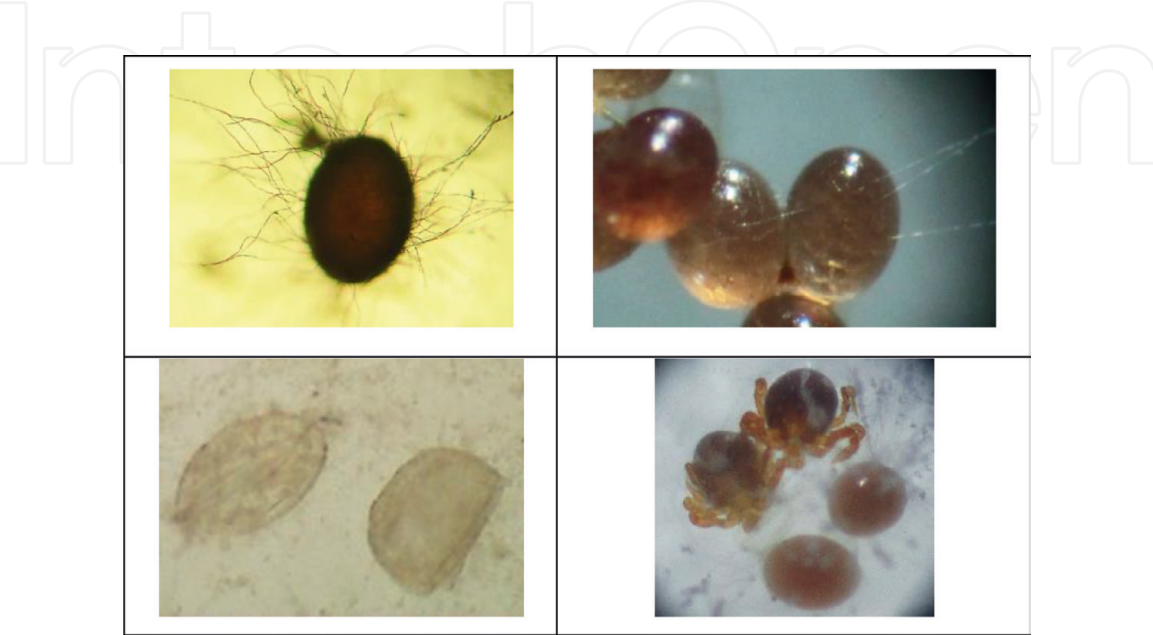


Figure 6. Hyphae of *M. circinelloides* grow and attach to the eggshells of ticks, penetrate and destroy them (COPAR archive).

sprayers. This would provide a solution to limit the risk of infestation in outdoor areas as waysides or the edge of grass along the roadsides, gardens, or even farms. Reduction in the presence of ticks in the soil also provides a sustainable and preventive tool to avoid damage to humans and animals.

2.3 Biofuel production

Some strains of several soil fungal species have been isolated according to their ability to convert fungal oils into esters, providing thus a sustainable way to obtain biofuel [48, 49]. The interest of microbial oils has increased as they are now used as commercial sources of several nutritionally important polyunsaturated fatty acids [50].

2.4 Health and soil fungal employment

Despite fungi being mostly considered responsible for fungal diseases that can range from nonsevere to mortal illnesses, fungal infections have become a serious health problem in immunocompromised patients largely.

Opposite to *Duddingtonia flagrans* and *Monacrosporium thaumasium*, the infection by *Mucor circinelloides* has been associated to clinical cases of mucormycosis, a sporadic and life-threatening infection caused by Mucorales. These are fungi distributed far and wide in the environment, in particular on woody surfaces and soils, where it can be easily isolated [27].

Several reports indicated nosocomial infection by *M. circinelloides* among immunocompromised people with skin wounds, or suffering diabetes mellitus [51].

Among animals, infection by *M. circinelloides* has been diagnosed in one Vietnamese potbellied pig presenting clinical signs of pneumonia, but information regarding the habitat or the level of inbreeding has not been provided [52].

Until now, long-term assays comprising the frequent administration (daily or twice a week) of a blend of spores of *M. circinelloides* and *D. flagrans* have been developed in pasturing horses. One group of seven horses received daily pellets containing the fungal spores during 64 weeks, and no adverse effects regarding respiratory, digestive, reproductive, or cutaneous damage were recorded [7]. Other group of eight horses was given pellets twice a week with the spores for a 1-year period, and after testing the activity of the respiratory, digestive, and reproductive systems, no alterations were recorded [41]. No signs of damage on skin integrity were observed.

Until now, there have not been reported any problem with people producing and managing spores/mycelium for longer than 10 years.

2.5 Conclusions

Inasmuch as STHs are transmitted through soil, it seems essential to develop measures on the environment to avoid reinfection, and the abusive administration of parasiticides. Some STHs originate from animals (domestic and wild), and helpful actions to reduce the risk of transmission are also required. Besides public education and hygienic behaviors, other activities should be applied to limit the presence and survival of infective stages of parasites. There have been described several species of soil fungi antagonists of eggs or larvae of helminths and ticks. Although several cases of disease have been linked to soil fungi, the absence of disease among people managing them or among animals receiving fungal structures seems to reinforce their safety, unless the patients are immunocompromised. The use of soil fungi against infections transmitted across ground gives a sustainable

measure to prevent damage to persons and animals, and might allow us to limit the administration of antiparasitic drugs to imperative situations only.

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Conflict of interest

All authors declare the absence of any financial or personal interests that could inappropriately influence or bias the current work. The final chapter has been approved by all the authors.

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