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Nemathophagus Fungi for Biological Control of Domestic Animal Parasitosis: A Review

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Abstract: Gastro-intestinal parasitism constitutes one of the greatest disease problems in grazing livestock Worldwide. Control of these parasites is now becoming a serious concern, due to the widespread and rapid development of resistance to chemotherapy this is largely the result of a more-or-less complete reliance on anthelmintics for worm control. In addition to development of resistance to chemotherapy, inadequate availability and high cost of commercial anthelmintic are the other important constraints of helminthes control in developing countries. Especially environmental concerns by use of chemical pesticides have increased the need for alternative method in the control of parasitic nematodes. Biological control is considered eco-friendly and a promising alternative in pest and disease management. Fungal biological control is a hopeful research area and there is constant attention in the use of fungi for the control of nematodes. Nematophagous fungi, also called as microfungi, Nematodes can be caught, killed and digested by them. In order to capture nematodes, they use specialized mycelial structures known as "traps, " "spores, " or "hyphal tips, " which assault nematode eggs and cysts before penetrating the nematode's vermiform cuticle, invading and digesting them. For example the fungus Duddingtonia flagrans has the capacity to survive passage through the gut, grow rapidly in faeces and feed on hatched parasitic larvae. In this review, biological control ability of nematophagous fungi is discussed.

Key words: Anthelminthic · Nematophagous-Fungi · Biological-Control · Drug-Resistance · Environment

INTRODUCTION

Since the beginning of human animal domestication, parasites have caused financial losses. Farmers and ranchers with herds infested with parasites suffer greater expenses in caring for sick animals and lose out on revenue due to decreased productivity. Some of the most widespread parasites in the world are helminthes. The two primary animal groups to which they belong are the roundworms or Nematoda and the flatworms or Platyhelminthes (flukes and tapeworms) [1].

There are two phases in the life cycle of gastrointestinal parasitic nematodes, which are significant worms in animals. These growth stages are external and endogenous. The exogenous stage is expelled from the animal and develops into eggs and larvae in the faeces, while the endogenous stage is parasitic in the gastrointestinal tracts (GT) of animals [2].

In order to control nematodes more efficiently, it is important to interfere with their entire life cycle that is, to reduce the numbers of nematodes in animals and on the pasture at the same time.

An exciting development in parasite control over recent years has centered on biological control of gut-worm parasites. For example the fungus *Duddingtonia flagrans* has the capacity to survive passage through the gut, grow rapidly in faeces and feed on hatched parasitic larvae [3]. Nematophagous fungi, also called as microfungi, are nematodes primary predators. Nematodes can be caught, killed and digested by them [4].

In order to capture nematodes, they use specialized mycelial structures known as "traps, " "spores, " or "hyphal tips, " which assault nematode eggs and cysts before penetrating the nematode's vermiform cuticle, invading and digesting them. Based on their

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saprophytic/parasitic ability, nematophagous fungi can be categorized in to three primary types although it consists of more than 200 species of fungi; nematod trapping fungi, endoparasitic fungi and egg- and cyst parasitic fungi. The first two types fight vermin by attaching to living nematodes with specialized structures and the third type, i.e Egg-and cyst parasitic fungi, uses their hyphal points to attack developmental stages of the nemathods [4].

Therefore, the objective of this paper is to review the extent of helminthophagous fungi for the biological control of animal helminth parasites.

Biological Control of Parisites: Biological control (BC) may be defined as the use of one living organism to achieve control over the targeted organism like parasite and thus reducing the population of pathogen below a thresholdwhere level it cannot cause clinical problems and/or economic losses in the animals. The philosophy behind BC is that by using one of the natural enemies of nematodes, it will be possible to reduce the infection level on pasture to a level at which the grazing animals avoid both clinical and subclinical effects due to parasitic [5]. Of all possible antagonistic organisms' only nematophagous fungi, earthworms and dung beetles have realistic potential as biological control agents. Besides these, a nematophagous fungus is finest option to biological control [6].

Overview of Nematophagous Fungi: Nematophagous (nematode-destroying) fungi comprise more than 200 species of taxonomically diverse fungi that all share the ability to attack living nematodes (juveniles, adults and eggs) and use them as nutrients. The fungi differ in their saprophytic/parasitic ability. While many of the trap-forming and egg-parasitic fungi can survive in soil saprophytically the endoparasites are mostly more dependent on nematodes as nutrient [7].

The ability to capture nematodes is connected with a specific developmental phase of the fungal mycelium. The trapping (predatory) fungi have developed sophisticated hyphal structures, such as hyphal nets, knobs, branches or rings, in which nematodes are captured by adhesion or mechanically. The endo parasites, on the other hand, attack nematodes with their spores, which either adhere to the surface of nematodes or are swallowed by them [8].

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. A prior emphasis on 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 [2].

Nematophagous fungus can be divided into three groups. These are nematode-trapping/ predatorial, Opportunistic or ovicidal and endoparasitic. The fungi of the first and second groups produce modified hyphae called traps, they bind and digest nematode larvae and eggs by a mechanical/enzymatic process. [9]. 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 chlamidospores germinate in the feces, forming a network of hyphae with the ability to capture and destroy infective forms of animal parasitic helminthes [10].

However, in recent years, biological controls become an important research area in the control of helminths due to the absence of the disadvantages of chemical controls and conformity to the goals of ecological sustainability. Predatory and ovicidal, nematophagous fungi have been shown in extensive in vitro and in vivo tests to effectively reduce recurrent infections by GINs in domestic animals [11].

Ecology of Nematophagous Fungi: Nematophagous fungi have been found in all regions of the world, from the tropics to Antarctica. They have been reported from agricultural, garden and forest soils and are especially abundant in soils rich in organic [13]. In Ethiopia isolates of four genera and six fungal species were obtained from three different soil samples (dung, forest and agricultural soil samples) taken from three different agro-ecological zones (Debre-Berhan, Bishoftu and Awash). Four genera identified were *Arthrobot*-rys, *Paecilomyces*, *Monacrosporium* and *Harposporium*. *Arthrobotrys* was the most widely isolated genera. All the four genera prevail in all agro-ecologies. *Arthrobotrys* were more prevalent in soil samples from Awash and Bishoftu areas, which represent lowlands and mid-altitudes respectively [14].

Nematode–Fungus Interaction Mechanisms

Recognition and Host Specificity: The process by which nematophagous fungus identify their prey is a

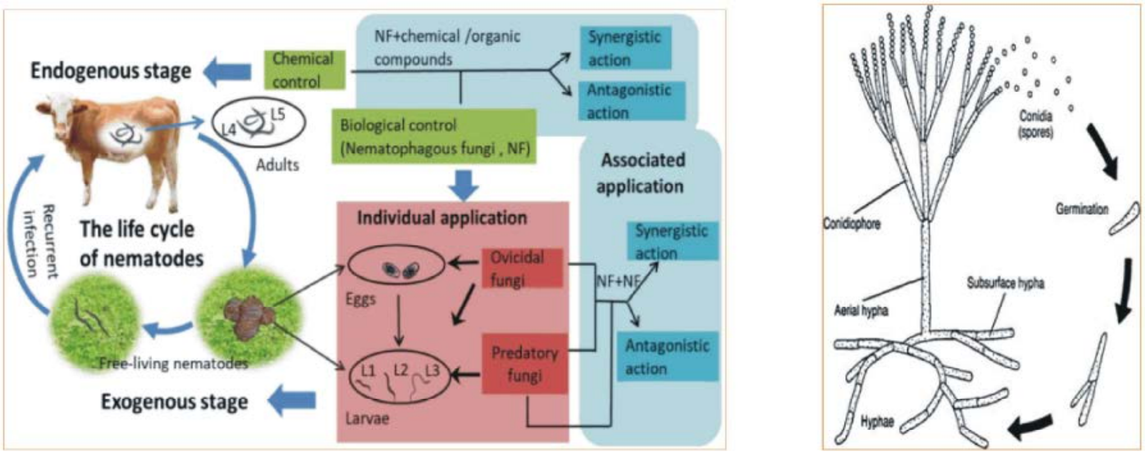

Fig. 1: Nematode and Fungal Life Cycle
Source: Al Ani, [12]

complicated one. None of the nematode-trapping species have been shown to exhibit simple host specificity; however endoparasite investigations have shown a slightly greater level of host specificity. However, it seems that cell-cell communication involves recognition processes [15].

During various stages of the fungus-nematode interaction, this could cause a specific physiological, morphological, or biochemical reaction. Nematodes are drawn to the fungi's mycelia, where they may cause the construction of traps and they are drawn even more to spores and completely formed traps. This is followed by a 'short range' or contact communication: adhesion. This phase may involve an interaction between a carbohydrate-binding protein (lectin) in the fungus and a carbohydrate receptor on the nematode. Recognition of the host is maybe also important for the subsequent steps of the infection, including penetration of the nematode cuticle [16].

Attraction: The morphology and consequently the saprophytic/parasitic ability of the fungi strongly influence the attractiveness of the fungi; nematodes are attracted to compounds released from the mycelium and traps of nematode-trapping fungi as well as to endoparasite spores. The more parasitic the fungi, the stronger the attraction; that is, the endoparasitic species infecting nematodes with conidia are more successful in drawing nematophagous fungi nematodes than the more saprophytic species with various trapping devices [11].

Adhesion: Under an electron microscope, nematodes can be seen contacting and adhering to the spores and traps of nematophagous fungi. Before contact with the nematodes, the extracellular fibrils in *Arthrobotrys oligospora* envelop the three-dimensional nets. Following contact, these fibrils orient themselves perpendicular to the host surface, most likely to aid in the nematod's subsequent fungal penetration and attachment [17].

Whether contact with the nematode has been formed, the endo parasite *Duddingtonia. coniospora* exhibits an entirely new kind of adhesive that appears to be made of radiating fibrils. By adhering particularly to the sensory organs at the apex of the worm's head, the *D. coniospora* spores prevent nematode attraction. Although the precise chemical makeup of nematophagous fungi's surface fibrils is unknown, they do contain proteins and polymers that contain carbohydrates [11].

Penetration: The fungus differentiates as a result of the traps' attachment to the worm. The nematode cuticle is punctured by a penetration tube that arises in *A. oligospora*. This step most likely involves the action of hydrolytic enzymes that dissolve the cuticle's macromolecules as well as the action of a mechanical pressure produced by the fungus that is entering and developing. The majority of the proteins in the nematode cuticle, including collagen, are hydrolyzed by a number of proteases that have been identified from nematophagous fungus [18].

Constricting Rings: While less research has been done, the patterns of nematode infection of other predatory fungus that employ sticky layers to trap nematodes (nets, hyphae, or knobs) seem to be somewhat comparable to those found for *A. oligospora*. On the other hand, constricting rings have a whole different mechanism of trapping. The three cells that make up the

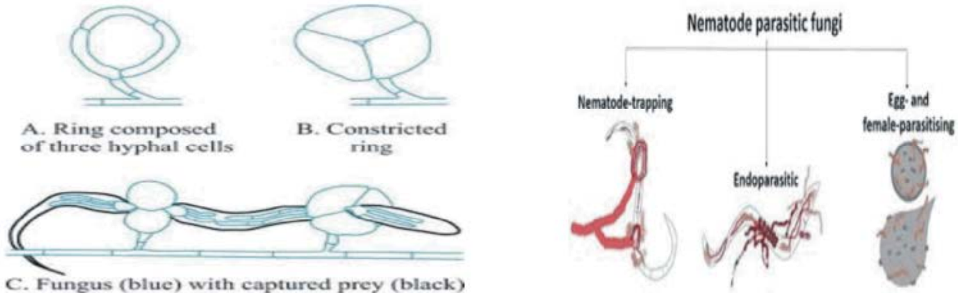

Fig. 2: Nematode–fungus Interaction
Source: Al-Ani, [12].

ring rapidly grow inward and close around the nematode when it travels into it. This is the result of a response set off by the nematode. Other stimuli, such as touch by a needle of the inside (luminal) surface of a ring, or heat, can also trigger the closure of the trap [16].

A detailed mechanism for closing the constricting rings is unknown. According to electron microscopy, the outer cell wall of the ring cells ruptures along a specific line on the inner surface of the ring during the ring-cell growth. It has been proposed that this wall pressure release will cause the ring cells' elastic inner wall to expand after a quick uptake of water. In *A. dactyloides*, the signal transduction route responsible for the ring cell inflating has been studied [19].

Most Promising Helminthophagous Fungi

Predatory Fungi: A vital tool for eliminating nematodes is a predatory fungus. They create modified hyphae known as traps, which can be non-adhesive (non-constricting or constricting rings) or adhesive (network, hyphae, branches and knobs) [20]. By means of mechanical or enzymatic processes, these traps bind and digest nematodes, both adult and larval [21]. This group of fungi only showed physiological effects; hyphae adhered to the eggshells of helminths without egg destruction. Therefore, the use of these fungi may have low efficiency, because undamaged eggs can cause recurrent infections [22].

However, no doubt that the effect of predatory fungi on the larvae of gastro intestinal nematodes is satisfactory. According to the most recent taxonomic classification, all predatory orbiliaceous fungi are assigned into three genera *Arthrobotrys*, *Drechsleralla* and *Dactylellina* based on the use of trapping devices as the primary criterion for generic delimitation [20].

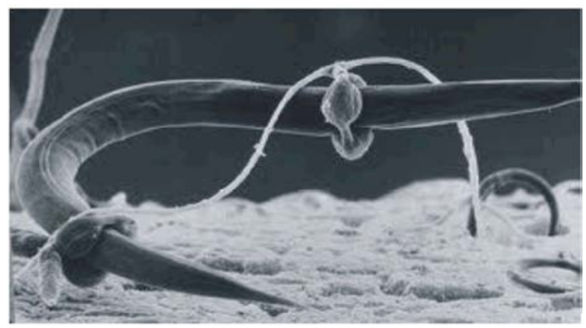

Fig. 3: Nematode trapping fungi
Source: Birgit, [4].

Duddingtonia Flagrans: The fungus *D. flagrans* acts as the main predatory fungus of gastrointestinal parasitic nematode larvae. The genus *Duddingtonia* possesses an outstanding characteristic in that it produces numerous chlamydospores, a type of thick-walled spore [23]. These spores have a shape that can range from elliptical to ovoid with a median septum [24]. The production of abundant chlamydospores is an advantage, because abundant fungal spores are an important strategy for survival and spread. Consequently, this group of fungi is more successful than other genera in the control of nematodes [25].

Duddingtonia flagrans is one of the most widely studied and most promising species. Its chlamydospores can withstand gastrointestinal transportation and other undesirable environments to germinate, forming as a predator device a three-dimensional network structure to capture living larvae in animal feces [24].

Experimental studies *in vitro* have shown that *D. flagrans* could reduce up to 96.4% of GINs, better than *Monacrosporium thaumasium* and *Arthrobotrys robusta* and in vivo tests a reduction of 55.15%–98.82% has been reported [26].

Arthrobotrys: Arthrobotrys is a typical genus of NF and was the first discovered in the 19th century [27]. This genus is characterized by a high ability to produce conidia and chlamydospores; *Arthrobotrys* has been deemed one of the most important genera to be used as a potential biocontrol agent among the predatory fungi to date. Conidiophores of species in this genus are typically simple or sparingly branched, bearing apical clusters of conidia [28].

Arthrobotrys reduced 80%–99% of GINs in in vitro studies. Although these species of the genus showed high efficiency for trichostrongylides, the percentage reduction of nematodes was different at the same dose. In addition to these reduced the larvae of *Haemonchus* spp. by up to 90%. NF can be made into edible pellets, mixed with grass and then fed into the GT through animal chewing. During this series of processes, chewing causes mechanical damage to NF and GT physical and chemical factors also have an impact on them [29].

However, in vivo experiments have proven that several species of *Arthrobotrys* can reach animal feces smoothly through the GIT and function successfully. In contrast to *D. flagrans*, many species of *Arthrobotrys* have shown effectiveness against trichostrongylides and cyathostomes in various livestock [30].

Monacrosporium: Fungi of the *Monacrosporium* genus have a well-developed ability to produce chlamydospores and form traps on conidia. The genus is defined by a single conidium produced on each tip of the conidiophores [20]. Importantly, the genus can still survive and maintain nematicidal ability after passing through the GIT of animals, which is a pre requisite for fungi to act on nematodes in feces. Mo. thaumasium has been successfully used in laboratories and under field conditions in the control of GINs in domestic animals [31].

Ovicidal Fungi: Ovicidal fungi are common soil saprophytes, the majority of isolated ovicidal fungi have been found to belong to *Humicola*, *Pochonia*, *Martiellera*, *Paecilomyces* and *Fusarium* [9]. Over the years, *Pochonia* has been treated as the most representative genus, with significant ovicidal action reported for GINs. Unlike predatory fungi, this group of fungi cannot form trapping devices [29]. Their hyphal penetration and internal egg colonization operates via a mechanical/enzymatic process, with morphological changes in the eggshell and embryo observed. However, the larvicidal activity of ovicidal fungi is rarely evaluated [32].

Pochonia: In the ovicidal fungal group, this species is used to form dictyochlamydospores and has been extensively studied as a biocontrol agent *Po. chlamydosporia* selectively parasitizes the eggs of gastrointestinal helminths. Its cannot only colonize the surfaces of eggs, but also penetrate into the insides of the eggs in the process of fungal action on nematodes [17].

Pochonia chlamydosporia has been used as the most common ovicidal fungi to control GINs in various domestic animals and it has been shown to reduce nematode eggs by 87.4% in in vitro tests Although the ovicidal activity of *Po. chlamydosporia* has been frequently evaluated, it is not known whether it has destructive power against larvae [33].

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*. It is a filamentous saprophytic fungus with action against trematode eggs (*Faciola hepatica*) [17]. 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 [34].

Mucor circinelloides, a soil filamentous fungus, is able to destroy nematode eggs in the feces of infected animals. The spores that colonize the animal feces germinate out a mycelium which penetrates the eggshell, invades the interior and damages both the eggshell and the embryo. Furthermore, it can survive in the digestive tracts of animals without loss of biological activity, thus providing a very helpful tool to prevent infection by ascarids among pasturing animals [31].

Other: It has been reported that other genera of NF, including *Paecilomyces* and *Mucor*, also have ovicidal action. *Paecilomyces lilacinus* is a common hyphomycete which has proven efficiency on the eggs of gastrointestinal parasite nematodes and tapeworms in ruminants. It has been found to be able to reduce the number of nematode eggs in the feces of dogs and horses but the effect was not as good as that of *Po. chlamydosporia* [35].

The use of independent predatory and ovicidal fungi is effective for the treatment of GT parasitic nematodes in a variety of domestic animals (including sheep, cattle, goats, horses, pigs and chickens) [32].

Table 1: *In vivo* and *in vitro* nematicidal tests with nematophagous fungi

		Nematicidal Activities (%)		
Fungi	Dose	*In vivo* test	*In vitro* Test	Reference
A. cladodes	1g pellets/10kgBW, twice aweek 5x10^5 spores/kgBW; 2 mL fungal suspension	52-59	68.7	[28]
D. flagrans	5 x 10^5 spores/kg BW, twice a week	85.4,	62.12-99.88	[26]
Mo. thaumasium	3 g of pellets/10 kg BW	79	-	[33]
Mo. thaumasium	1ml of solution containing 1000 spores, single dose	-	95	[32]
Po. chlamydosporia	1.5 x 10^5 conidia	64-86	26-67	[17]
Mu. circinelloides	1 mL pellet, 2 x 10^6 spores/m	61-67	53	[25]

Differences in the NF trapping efficiency of nematodes are universal among different species of the same genera and different isolated strains of the same species. Two factors account for these differences; internal factors include the cuticular nature of parasitic nematodes and the antigenic variations in the different species of nematodes or different isolates of the same species of fungus [33]. Extrinsic factors include the number of nematodes available and environmental factors, for example, a low density of nematodes being insufficient to stimulate NF to produce trapping structures, resulting in a low predation efficiency of NF [26].

Potential New Strategies of Using of Netmathophagus Fungi as a Biological Control: The use of a combination of biological controls or mixed biological and chemical controls may reduce the flaws evident in individual administration and it may even enhance fungal predation ability [26]. However, there are compatibilities or incompatibilities (the combined agents can produce compounds that inhibit each other in a joint application) among some fungi, predators and compounds and an incompatibility can seriously prevent the combined strategy from controlling nematodes [36].

Coadministration of Fungi with Fungi: Studies showed that the methods of associated application among fungi can be summarized into three forms: a combination of two or three fungi, a combination of different groups of fungi (predatory and ovicidal) and a combination of identical groups of fungi (predatory fungi). In combined administration, these fungi are required to meet two basic requirements: first, they are required to have the ability to pass through the animal's GT without losing vitality, as if they were administered alone; second, they must not inhibit each other's growth [37]. The associations between identical groups of fungi (*D. flagrans* + *Mo. thaumasium*) or different groups of fungi (*A. cladode* + *Po. chlmydosporia*, *D. flagrans* + *Po. chlamydosporia*, *Mo. thaumasium* + *Po. chlamydosporia*) have been shown to have synergistic effects, which significantly enhance predation efficiency compared to using a single fungus alone [38].

Coadministration of Fungi with Compounds: Researchers found that the combination between chemical drugs and NF seems to be effective [39]. From the perspective of the predation target, this combination could disrupt the whole life cycle of nematodes, but there have been relatively few successful cases of doing so. However, organic compounds could act as efficient vehicles in the topical administration of NF [40].

Reports of synergistic effects for the simultaneous administration of chemical drugs and NF are scarce [38]. Some researchers reported that the combination of *D. flagrans* and levamisole hydrochloride was feasible, with a significantly lower EPG recorded for their combination than for exclusive administration of the chemical drug. Nevertheless, most studies suggest an antagonistic effect between chemical drugs and NF. For example, the antiparasitic compounds albendazole, ivermectin and levamisole have been found to have a negative impact on the viability of *A. oligospora, Pa. lilacinus* and Arthrobotrys spp [41]. *Duddingtonia flagrans* has Duddingtonia been found to have high susceptibility to tested drugs, including fenbendazole, thiabendazole and ivermectin, carbendazim and difenoconazole. *In vivo* tests also confirmed that chemical drugs inhibited the activity of *D. flagrans* spores when used in combination with albendazole [25].

On the other hand, numerous studies have incorporated fungi into a sodium alginate matrix and produced edible pellets, which helps fungi overcome undesirable factors to successfully pass through animal GTs and contributes to the long-term preservation of spores [42]. Dimethyl sulfoxide as a permeabilizer and mineral oil as an adjuvant assisted in an excellent penetration and adhesion of conidia to the nematode epidermis [40].

Importantly, this method presented a high affinity for conidia fungi and effectively acted on *Rhabditis spp*[16]. These findings were all confirmed under laboratory conditions, except for the compatibility of fungi, which was confirmed with an antiparasitic *in vivo* test [25].

Fungi-Nematode Interaction for Diversity and Ecology: Ecosystems consist of many types of organisms, including deferent types of microscopic organisms such as bacteria, protozoa, fungi and small animals such as nematodes. Together, these organisms interact with each other and with macroscopic organisms such as plants and large animals to perform ecosystem functions. Their interactions happen in multiple ways, can be direct or indirect, involving two or more partners and occur through deferent mechanisms such as predation, parasitism, mutualism, or competition. These interactions are critical for maintaining ecosystem balance [43].

Nematodes do not decompose organic matter but instead are parasitic or free-living organisms that feed on living materials. On the other hand, fungi are the principal decomposers of dead organic matter; they perform fundamental roles in nutrient cycling in the ecosystem. Helminthophagous fungi can also produce ecological silver nanoparticles. They convert toxic metal ions into non-toxic nano particles through their catalytic effect [44].

CONCLUSIONS AND RECOMMENDATIONS

Gastrointestinal nematodes remain a major threat to the health and welfare of ruminants all over the world having severe consequences on the animal as well as the livestock leading to economic loss and restricted productivity. Anthelmintic has been used as the major option to control this pathogenic nematode which has resulted in parasitic drug resistance and affect environment. Thus the different mechanisms of the different groups of Nemathophagus fungi, may contribute to the positive effects of controlling of parasites. In this review it is understood that there are most promising nemathophagus fungi enabling the biological methods of parasitic control is effective.

These include, the predatory fungi (like *Duddingtonia flagrans*, *Arthrobotrys*, *monacrosporium*); ovicidal fungi (like *Pochonia*, *mucor circinelloides*, *paecilomyces* and *mucor*) and others which are still under study. It is also understood that the combination of these nemathophagus fungi together with chemicals and other nemathophagus fungi can create synergestic effect to control animal parasites. Depending upon the above conclusion the following recommendation is forwarded;

- Fungal biological control is a hopeful research area and there should be constant attention in the use of fungi for the control of nematodes.
- Bioaintegrated nematode parasite control programmes for grazing livestock should be implemented.

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