Review on The Use of Nematophagus Fungi as biological control of nematode of livestock

Tolessa Ebissa and Dawit Hora

Department of Veterinary Medicine, Asossa University, Asossa 35099, Ethiopia
Email address: kuletole@gmail.com, Cell phone: +251921889089

Summary: In nature, abundant natural antagonists of helminthes are found which including fungi, bacteria, viruses, insects, mites and some invertebrates that have been found potential to prey or invade of helminthes. Fungi are considered as the major microbial organism in many soils that have a significant association with nematodes by constantly destroy nematodes in nearly all soils at different geographical areas to fulfill their nutritional requirements. Among these fungi, nematophagous or nematode-destroying fungi are those that can capture, parasitize or paralyze nematodes and act as natural enemies of plant-parasitic and animal-parasitic nematodes. Therefore the objective of this paper is to review the use of Nematophagus fungi as biological control for nematodes in livestock and to highlight different types of nematophagus fungi and their mechanisms of action. Nematophagous fungi are cosmopolitan microorganisms able to modify their saprophytic behavior to carnivorous which enables them to act as natural enemies of nematodes. Depending on their mode of attacking mechanisms nematophagous fungi are divided into four groups as nematode-trapping, endoparasitic, egg and female-parasitic and toxin-producing fungi. These groups of fungi are used as best option as biocontrol where currently the problem of anthelminthic resistance is increasing since they decrease the level of nematode free-living stages in the soil ecosystem. Nowadays it is becoming an important non-chemical option for controlling gastrointestinal nematode in animals since biocontrol agents can control a target organism by reducing its population to a level that no longer causes clinical problems and economic losses.

[Tolessa Ebissa and Dawit Hora. **Review on The Use of Nematophagus Fungi as biological control of nematode of livestock.** *Biomedicine and Nursing* 2019;5(3): 72-82]. ISSN 2379-8211 (print); ISSN 2379-8203 (online). http://www.nbmedicine.org. 10. doi:10.7537/marsbnj050319.10.

Keywords: Biocontrol, livestock, Nematode and Nematophagus fungi.

1. Introduction

In nature, abundant natural antagonists of helminthes are found. these large number of organisms including fungi, bacteria, viruses, insects, mites and some invertebrates which have been found potential to prey or invade of helminthes. Fungi are considered as the major microbial biomass in many soils that have a significant association with nematodes in rhizophere and thus, they can constantly destroy nematodes in nearly all soils at different geographical areas (Masoomeh *et al.,* 2004). Among these fungi, nematophagous or nematode-destroying fungi are those that can capture, parasitize or paralyze nematodes and act as natural enemies of plant-parasitic and animal-parasitic nematodes (Ya-Juan Xue *et al*., 2018). And this ability of nematophagus fungus has increased the value of these organisms as potential candidate as bio-control for plant, human and livestock nematodes (Dijksterhuis *et al.,* 1994, Waller and Faedo, 1996 and Masoomeh *et al.,* 2004**)**

There are over 700 nematophagous fungal species, from several phyla, such as the Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota. Moreover, even organisms belonging to the phylum Oomycota have had their nematophagous activity as described by (Li *et al.,* 2015; Filippe, 2018) These fungi have several characteristics including highly specific parasitism to nematodes compared to plants and animals thus they are less or not specific parasite to plants and higher animals and growth at suitable pH and temperature ranges on natural or synthetic media **(**Masoomeh *et al.,* 2004).

Nematophagous fungi are cosmopolitan microorganisms able to modify their saprophytic behavior to carnivorous which enables them to act as natural enemies of nematodes in which they have developed highly different strategies to infect and allowing them to feed on nematodes under unfavorable nutritional conditions (Braga and Araújo, 2014; Degenkolb and Vilcinskas, 2016; Filippe, 2018). Nematophagous fungi colonize soils rich in organic matter under different temperature and humidity conditions, thus contributing to the biological equilibrium of the soil by interacting with local micro flora and micro fauna. They are usually isolated from soil and faeces from different animals **(**Saumella *et al.,* 2015**).** They are divided into four groups depending on the mechanism of action they imposed on the nematodes as endoparasitic fungi, opportunistic fungi, toxic fungi, and nematode-trapping fungi (Ya-Juan Xue *et al*., 2018**).**

Nematophagous fungi have been seen as potential biological control agents against nematodes for a long time. First attempts were made in the 1930s to reduce plant-parasitic nematodes in soils (Larsen, 2000). During the following 50 years, the research using nematophagous fungi to control nematode infections were only sporadic. In the 1980s, the research was reinforced and trials begun feeding livestock with spores of nematophagous fungi. Some research study previously were done show that especially the nematode-trapping fungus like *Duddingtonia flagrans* was effective in reducing the worm burden in livestock infected with nematode **(**Andersson, 2013). In addition to control plant nematodes, several commercial biological nematicides created from nematophagous fungi have been developed to date because of the development of anthelmintic and pesticide resistance of the helminthes is increasing (Vieiraa *et al., 2019).*

The widespread resistance of gastrointestinal helminthes to different compounds has led to an urgent need for novel technologies to prevent and control parasitic diseases in livestock. The selection of new anthelminthic and pesticides has focused its attention to programs with low toxicity to humans, animals and wildlife, low environmental impact, low residues in animal by-products and high compatibility with integrated parasite management. To meet the expectations in applying these programs, new methods to control gastrointestinal helminthes in animals have been conducted, with emphasis on alternative strategies to control nematodes with nematophagous fungi (Liu *et al.,* 2015).

In the prevention and control of parasites in livestock, the current problem is that the traditional use of chemical anthelmintic drugs to control gut parasites has resulted in resistance to these drugs (Sargison 2012, Falzon *et al.* 2013; Ya-Juan Xue *et al*., 2018**).** The biological control of parasitic helminths of animals through the use of nematophagous fungi presents satisfactory results in several studies and put as use of these organisms is an important in control and prevention of nematode infection in animals (Oliveira *et al.,* 2018a, Vieiraa *et al.,* 2019). Therefore the objective of this paper is/are:

- ✓ To review the use of Nematophagus fungi as biological control for nematodes in livestock.
- ✓ To highlight different types of nematophagus fungi and their mechanisms of action.

2. Biology Of Nematophagus Fungi

Nematophagous fungi comprise several species and exist in all regions of the world, from tropics to the Antarctica. They are commonly found in soils that are rich in organic substance. The density of nematophagous fungi is highest in the upper 20 cm of the soil horizon and very few are found below 40 cm of depth of the soil (Persmark *et al*., 1996). Most nematophagous fungi can grow both as saprophytes by decaying organic matters as substrates, and as parasites using nematodes as their source of nutrients and those which can living in nitrogen limiting habitats have an advantage over other fungal saprophytes because of they can use nematodes as an important source of nitrogen **(**Andersson, 2013).

Depending on their mode of attacking mechanisms of nematodes, the nematophagous fungi are divided into four groups as nematode-trapping (formerly sometimes called predacious or predatory fungi), endoparasitic, egg and female-parasitic and toxin-producing fungi (Lopez-Llorca, 2008).

Nematophagous fungi can undergo a process of hyphal differentiation into adhesive trap structures. For all trap forming fungi, this process can be induced by external stimuli, such as the presence of nematodes substances derived or excreted by them in adverse conditions such as water and/or nutrient shortage or spontaneously in some species. According to Nordbring-Hertz *et al.* (2006), the nematode-trapping fungus like *A. oligospora* can be induced to form traps by the presence of small peptides, such as the phenylalanyl valine, with high proportion of nonpolar and aromatic amino acids or their amino acid components in combination with low-nutrient conditions or nutrient shortage in both liquid and solid media (Maciel, 2009**).**

The nematode predating process starts when the fungus attracts the nematodes with traps, organic and inorganic substances such as CO2, ammonia and sialic acid and then captures them in the traps. After the capture, regardless of the trap type, the fungus penetrates the nematode and develops inside it, consumes its content and its vegetative and reproductive structures emerge on the surface (Maciel, 2009).

The nematode-trapping fungi, as the name implies, capture nematodes with the aid of hyphal trapping devices of various shapes and sizes, as in adhesive three dimensional nets, adhesive knobs, non-adhesive constricting rings and non-constricting rings. A few nematode-trappers capture nematodes without visible traps in an adhesive substance formed on their hyphae, as in case of *Stylopage* spp (Lopez-Llorca, 2008).

The egg and female-parasitic fungi infect nematode females and the eggs they contain, using appressoria or zoospores. Finally, the toxin-producing fungi immobilize the nematodes by a toxin, prior to hyphal penetration through the nematode cuticle. In all four nematophagous groups, nematode parasitism results in a complete prey or egg digestion, activity which supplies the fungus with nutrients and energy for continued growth (Lopez-Llorca, 2008).

Table1: Groups of Nematophagous fungi and their predation characteristics on nematode.

Groups	Predation characteristics on nematodes
Nematode-trapping	Produce modified hyphae called traps, with which, by a mechanical/enzymatic process, they bind and digest nematode larvae.
Opportunistic or ovicidal	Produce modified hyphae called traps, with which, by a mechanical/enzymatic process, they bind and digest eggs, cysts and nematode Female.
Endoparasitic	Use spores (conidia, zoospores) as infection structures, which may adhere to nematode cuticle or be ingested.
Toxin-producing	Secrete toxins that immobilize the nematodes, with posterior hyphae penetration through the cuticle and complete colonization of the nematode.
Producers of special attack Devices	Produce special attacking devices that cause mechanical damage to the nematode cuticle, resulting in extravasation of the inner nematode contents and allowing complete nematode colonization

Source: (Filippe, 2018)

3. Taxonomy And Phylogeny

Nematophagous fungi are found in most fungal taxa: Ascomycetes (and their hyphomycete anamorphs), Basidiomycetes, Zygomycetes, Chytridiomycetes and Oomycetes. It therefore appears that the nematophagous habit evolved independently in the different fungal taxonomic groups. It was suggested that the nematophagous habit evolved from lignolytic and cellulolytic fungi, as an adaptation to overcome competition for nutrients in soil (Lopez-Llorca, 2008).

Figure 1: Taxonomic position of nematophagous fungi with examples of genera.

Division	Genus	Interaction
chytridiomycete	Cateneria	EP and FP
Oomycetes	Myzocytium nematophthora	EP EP
zygomycetes	Stylopage cystopage	NT NT
Deuteromycetes	Arthrobotry Monacrosporium Dactylaria Dactylella Nematoctonus Drechimeria Verticillum Paecilomyces Fusarium Harposporum	NT NT NT NT and EggP NT and EP EP EP, EggP and FP EggP EggP and FP EP
basidomycetes	Hohenbuhelia (telomorph of nematoctonus) pleurotus	NT and EP NT and toxic
Ascomycetes	Atricordyceps (telomorph of harposporum)	EP

EP: Endoparasitic; NT: Nematode Traping; FP: Female Parasite; EggP: Egg Parasite.
Source: Dackman *et al.*,1992

Recently, the egg-parasitic fungi previously placed within the genus *Verticillium* were transferred to the new genus *Pochonia*, in parallel with entomopathogenic species of *Verticillium*, which were transferred to the genus *Lecanicillium* based on both morphological and molecular characters (Zare, *et al.,* 2001). The teleomorphs of the *Pochonia* species are located within *Cordyceps*. The best known species of egg parasites are *P. chlamydosporia* and *P. rubescens*, but species of other genera such as *Paecilomyces lilacinus* and *Lecanicillium lecanii,* are also known to parasitize nematode eggs (Lopez-Llorca, 2008).

4. Classification Of Nematophagus Fungus And Their Mechanism Of Action On Nematodes

Several microorganisms parasitize or prey upon nematodes, whose action is known as biological control, since they decrease the level of nematode free-living stages in the soil ecosystem (Chen and Dickinson, 2004). The nematode predating process starts when the fungus attracts the nematodes with traps or organic and inorganic substances such as CO2, ammonia and then captures them in the traps. After the capture, regardless of the trap type, the fungus penetrates the nematode and develops inside it, consumes its content and its vegetative and reproductive structures emerge on the surface (Mota *et al.,* 2003). These nematophagus fungi are classified in to four different types based on their action of predation as follows (Maciel, 2009**).**

4.1. Nematode Trapping Fungus

The nematode-trapping fungi, as the name implies, produce specialised hyphal trapping devices of various shapes and size such as; adhesive networks, knobs, and constricting or non-constricting rings. A few nematode-trappers capture nematodes without visible traps in an adhesive substance formed on their hyphae, e.g. *Stylopage* spp. Fungi in this class also produce nematode chemo-attractant and/or chemotoxic substances to capture, penetrate and destroy nematodes within short period of time. (Jansson *et al.,* 2006, Lopez-Llorca, 2008).

Many nematode-trapping fungi do not form traps spontaneously; instead they are dependent on external stimuli like living nematodes (Dijksterhuis *et al*., 1994). Already in the 1950s, a substance called nemin that could induce trap formation was extracted from culture filtrates of nematodes. Nemin was suggested to be a peptide of low molecular weight. Later, short peptides were shown to induce traps in *Arthrobotrys oligospora*. Recently, it was shown that ascarosides, which are constitutively secreted by nematodes, trigger trap formation in nematode-trapping fungi (Hsueh *et al.,* 2013). Ascarosides are composed of the sugar ascarylose linked to a fatty-acid chain. More than 100 different ascarosides have been identified from various nematodes (von Reuss *et al.,* 2012 and Andersson, 2013).

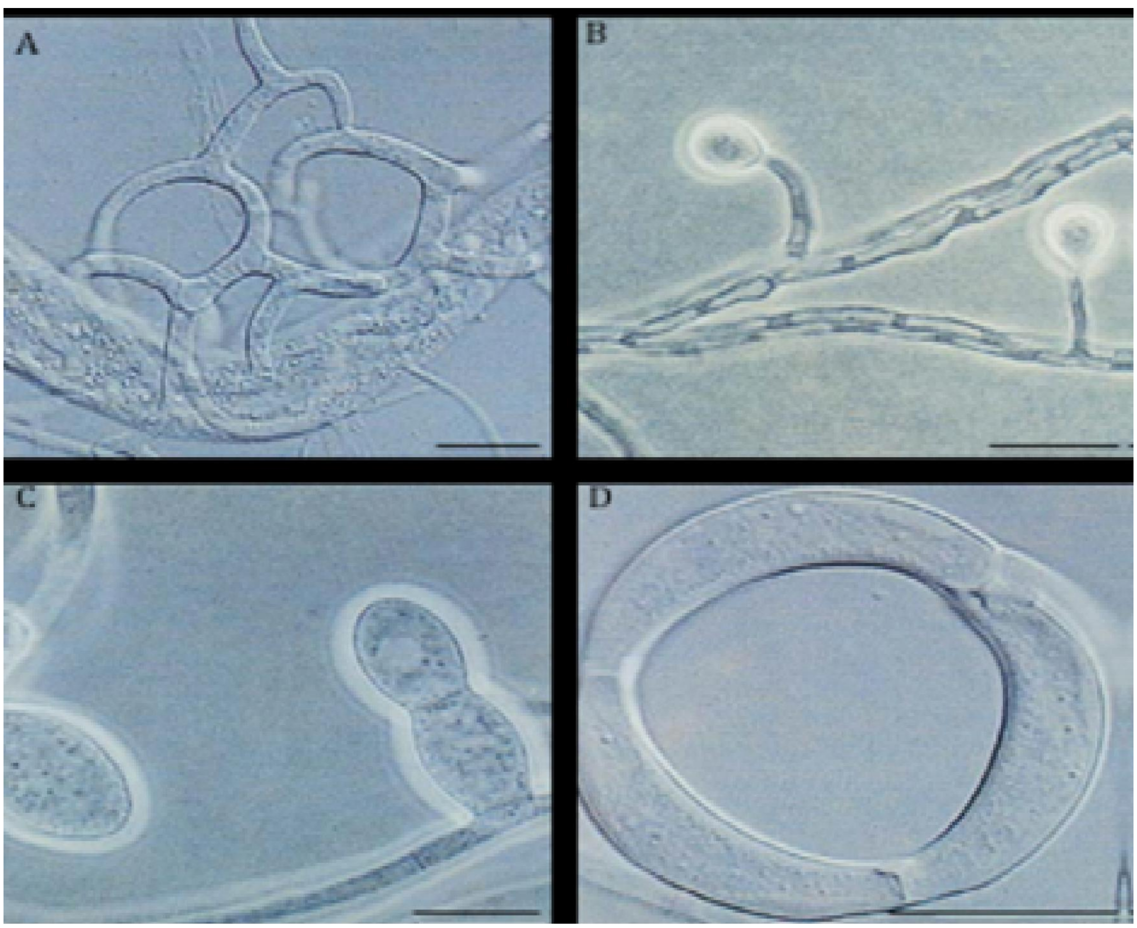

Figure 2: Major types of traps in nematode-trapping fungi.

The nematode-trapping fungi use different kinds of traps to capture and infect nematodes. Different species of the nematophagus fungus have different trap to enable them parasitize the nematode as in *A. oligospora* traps nematodes by an adhesive three-dimensional net. *M. haptotylum* develops at the apices of hyphal branches a structure called knobs. The knob is an adhesive single cell that can detach from the mycelia, travel along with the nematode and subsequently penetrate the nematode cuticle and infect the nematode. *Monacrosporium cionopagum* has adhesive hyphal branches that consist of one or more cells to infect nematodes (Nordbring-Hertz *et al.,* 2006).

Arthrobotrys dactyloides uses a mechanical trap called constricting ring to trap nematodes (Dijksterhuis *et al*., 1994, Nordbring-Hertz *et al*., 2006). This is a fascinating structure that consists of three cells. When a nematode enters the ring, the three cells inflate and capture the nematode. The closure of the trap is very rapid (0.1 s) and is triggered by physical contact between the nematode and the constricting ring cells. The closure is also stimulated by heat or by touching the luminal side of the ring with a needle (Andersson, 2013).

A fifth type is the non-constricting ring which is formed when an erect lateral branch of the vegetative hyphae thickens and curves to form a three cells ring that fuses with the stalk. The non-constricting rings are always accompanied with adhesive knobs (Liu *et al.,* 2009). In addition, a few nematode-trapping fungi such as *A. oligospora* and *A. dactyloides* form conidial traps. The conidial traps develop directly along the germination of the conidia, without an intermediate hyphal phase (Andersson, 2013)

A, adhesive net of Arthrobotrys oligospora (bar 20 μm); B, adhesive knobs of Monacrosporium haptotylum (bar 10 μm); C, adhesive branches of Monacrosporium gephyropagum (bar 10 μm); D, constricting ring of Arthrobotrys brochopaga (bar 5 μm) (Andersson, 2013).

4.1.1. Infection Mechanism

4.1.1.1. Attraction

Despite morphological differences in the trapping structures, the infection mechanism is similar between different nematode-trapping fungi (Dijksterhuis *et al.,* 1994). The infection mechanism starts with attraction. Since the fungi are non-motile in comparison to nematodes they need to attract the nematodes in some way. The mycelia and traps release compounds that attract the nematodes. The traps have a greater attractiveness than the vegetative mycelium. Furthermore, fungal species being relatively more parasitic show an increased attractiveness as compared to more saprophytic types (Nordbring-Hertz *et al.,* 2006). A volatile or a small and rapidly diffusing compound that is continuously produced by the fungus has been responsible for the attraction. Until recently, the exact components of these compounds have been unknown. However, chemical studies on the culture medium of the knob-producing *Arthrobotrys entomopaga* produce two compounds paganin A (colourless oil like substance) and blumenol that showed strong nematode-attracting abilities (Andersson, 2013).

4.1.1.2. Adhesion

The adhesion between the fungi and the nematode has been extensively studied during the years. Electron-microscopically analysis has shown that the traps of *A. oligospora* have a layer of polymers on their surface even before contact with the nematode. After contact there is an increased secretion of surface polymers and the fibrillar layer becomes oriented in one direction, perpendicularly to the orientation of the nematode. Gel chromatography showed that the surface polymers contain neutral sugars, uronic acids, and proteins, based on sugar inhibition experiment, it was suggested the infection process in *A. oligospora* was initiated by binding between a lectin present on the trap and a carbohydrate ligand found on the nematode surface. Subsequently, a N-acetylgalactosmine (GalNac)-specific lectin was isolated from *A. oligospora* (Andersson, 2013). In addition, some authors suggested that the adhesive coating, besides its role in adhesion, also serves as a matrix, harboring many extracellular virulent proteins (Liang *et al*., 2013) however, the molecular mechanisms of the attachment of the traps to the nematode cuticle are not yet known (Andersson, 2013).

4.2. Penetration

Following adhesion, the nematode-trapping fungi form a penetration tube that pierces the nematode cuticle which involves the use of both enzyme activities and mechanical pressure. The penetration site is effectively sealed to prevent leakage of nematode contents out into the environment. Nematode cuticle made from proteins which is mainly of collagens that makes it likely that the nematode-trapping fungi to penetrate the cuticle with aid of proteases. Indeed, different studies showed that many proteases have been isolated and characterized as in *A. oligospora* and other nematode trapping fungi which is a serine protease that could digest proteins present on the nematode cuticle (Dackman *et al*., 1992; Andersson, 2013)

In addition, a common feature for all nematode-trapping fungi is the presence of dense bodies inside the trap. These dense bodies are cytosolic and contain catalase and amino-acid oxidase activity which indicates that they are peroxisomal. Dense bodies are rapidly degraded after the formation of the infection bulb and it has been suggested that they contain material to facilitate the penetration and the initial development of trophic hyphae; (Dijksterhuis *et al.,* 1994; Andersson, 2013).

4.2.1.1. Degradation

Following penetration, the infection tube swells inside the nematode and form an infection bulb (Dijksterhuis *et al.,* 1994). Trophic hyphae develop from the infection bulb and the infecting fungus digests the nematode. Morphologically, both the infection bulb and the following trophic hyphae show the same characteristics as normal vegetative mycelium. However, the endoplasmic reticulum is highly proliferated in both structures. The time course for the infection process varies between different nematode-trapping fungi, and also depending on the nematode species being infected and even between different individuals of the same nematodes species. In *A. oligospora*, the process from adhesion until penetration and immobilization of the nematode usually takes 1-4 hour. The infection bulb is formed and trophic hyphae are developed thereafter 12-24 hour the growth rate of the trophic hyphae is retarded and growth of fungal mycelium outside the nematode is initiated. The infection process is usually completed within 48-60 hour (Dijksterhuis *et al.,* 1994 and Andersson, 2013)**.**

Table 2: Predatory Nematophagous fungi and their trapping mechanism

Predator	Trapping mechanism
Arthrobotrys robusta	Adhesive nets
Dactylaria gracilis	Constricting rings
Dactylella cionopaga	Adhesive braches
Dactylella ellipsospora	Adhesive knobes
Dactylella gephyropaga	Adhesive branches
Dactylella leptospora	Non constricting rings
Dactylella phymatopaga	Adhesive knobes
Dactylella stenobrocha	Constricting rings

Source: (Gray and Smith, 1984)

4.2. Endoparasitic Fungi

Endoparasitic fungi use their spores (conidia or zoospores) to infect nematodes. The propagules adhere to the nematode cuticle and the spore content is then injected into the nematode, or the spores are swallowed by the host. Most of these fungi are obligate parasites of nematodes and live their entire vegetative stages inside infected nematodes (Lopez-Llorca, 2008).

The endoparasites do not produce extensive mycelia but exist as conidia in the environment and infect nematodes by either adhering to the surface of the prey or direct ingesting. The conidia germinate rapidly and invade the entire nematode with assimilative hyphae absorbing all the body contents (Liu *et al.,* 2009). Endoparasites do not use hyphae for predation they rather use spores (conidia, zoospores) as infection structures, which may adhere to the nematode cuticle or be ingested (Braga and Araújo, 2014 and Filippe, 2018).

Table 3: Some of Endoparasitic nematophagus fungi and their mode of action

Endoparasites	Mode of action
***Myzocytium* spp.**	Encystment of motile zoospore
Acrostalagmus bactrosporus	Adhesive spores
Acrostalagmus goniodes	Adhesive spores
Acrostalagmus obo vatus	Adhesive spores
Cephalosporium balanoides	Adhesive spores
Harposporium anguillulae	Spores ingested
Harposporium helicoides	Spore ingested
Harposporium lilliputanum	Spore ingested
Spicaria coccospora	Adhesive spore

Source: (Gray and Smith, 1984)

4.3. The Egg and Female Parasitic Fungi

The egg and female-parasitic fungi infect nematode females and the eggs they contain, using appressoria or zoospores. The ovicidal group also uses traps in the process of predation. However, the target groups are eggs, cysts and nematode females (Filippe, 2018)**.** They have the ability to attack the egg stage and may have a role in the control of animal parasites which have a long development and/or survival time in the egg stage in the environment outside host, e.g., Ascaris, Fasciola spp., amphistomes etc **(**De and Sanyal, 2009**).**

4.3.1. Mode of Action

The infection of nematodes and their eggs by various nematophagous fungi follows a similar, general pattern. This is illustrated here by infection of nematode eggs by *Pochonia rubescens* and also by the zoospores of *Catenaria anguillulae*, which infect vermiform nematodes (Lopez-Llorca, 2008).

Penetration of nematode eggs by *P. rubescens* starts with contact of the hyphae with the egg and subsequent formation of an appressorium. An extracellular material (ECM) or adhesive, is formed on the appressorium, and is revealed by labelling with the lectin Concanavalin A (Con A), indicating that it contains glucose/mannose residues. From the appressorium the fungus penetrates the nematode eggshell by means of both mechanical and enzymatic components. The nematode eggshell contains mainly chitin and proteins and therefore chitinases and proteases play an important role during eggshell penetration (Jansson *et al.,* 2002). The ECM contains the protease P32 that can be immunologically detected using both fluorescent stains or/and colloidal gold. The proteolytic activity causes the degradation of eggshells (Lopez-Llorca, 2008).

The life cycle of *C. anguillulae* starts with uniflagellate zoospores which become attracted to natural orifices (mouth, anus, excretory pores, etc.) of nematodes. The flagellar movement is supported by the mitochondria at the base of the flagellum. Upon contact with the nematode cuticle the zoospores show an "amoeboid movement" before encystment takes place. During encystment a cell wall is formed covered by an adhesive, and the flagellum is withdrawn. The encysted zoospore forms an infection peg which penetrates the nematode cuticle. Within 24 hours the developing fungus invades and digests the nematode contents, and zoosporangia are formed from which the zoospores are released to infect new hosts. *Catenaria anguillulae* also has the ability to infect nematode eggs (Lopez-Llorca, 2008).

5. Use Of Nematophagus As Biological Control Of Nematode

Biological control of nematodes becomes an important, integrated element and sustainable strategies to control nematodes in livestock. Biological control defined as the activity of natural enemies which include classical, un-exploited organisms and genetically modified organisms (Larsen, 2006).

unlike other methods which are directed at the parasitic stages within the host biological control is targeted at the free-living stages on pasture focuses on the faecal deposits in which eggs, L1, L2 and L3 larval stages are found to reduce the number of infective stages that are available to be picked up by grazing susceptible individuals of the different species of livestock (Waller, 2006). Biological control of nematodes in livestock aims to establish a condition where grazing animals are exposed to a low number of infective larvae and reduce the level of nematode parasitism in livestock so that natural immunity in the animals will tolerate these low levels (Epe *et al.,* 2009).

Biological control is becoming an important non-chemical option for controlling GIN in animals since biocontrol agents can control a target organism by reducing its population to a level that no longer causes clinical problems and economic losses. In addition, biocontrol agents have low mammalian toxicity, high efficacy, naturally occurring and multiply to a level that matches its target organisms. Thus, biocontrol agents can avoid the issue of chemical residues in food and is an attractive option for organic farming (Larsen, 2006).

Among these biological control microorganisms, the nematophagous fungi are the most common organisms used for the control of nematode infection in animals. The major nematophagus fungi involved in parasitizing of nematodes are nematode trapping, endoparasitic and egg and female parasitic fungi as indicated in table 5. From these nematode trapping fungi are well studied which includes Arthrobotrys, Duddingtonia, Nematoctonus and Monacrosporium genera are able to capture, kill and digest animal parasitic nematodes, serving as potential biological control agents (Maciel, 2009).

Nematophagous fungus *Duddingtonia flagrans* has been studied as an alternative agent for the biocontrol of infective larvae of parasites in domestic animals. *D. flagrans* can produce many thick walled chlamydospores that survive the gut passage, germinate, develop, and produce predatory structures in feces to attack first-stage to third-stage larvae (L3) of parasitic nematodes before they spread to the herbage (Ya-Juan Xue *et al*., 2018).

Duddingtonia flagrans traps the free-living larval stages, which include eggs, L1 and L2 stages within the faecal deposit, and infective third-stage larvae on pastures. D. flagrans can survive passage through the gastrointestinal tract in the host and might be effective in trapping larvae present in faeces, thereby reducing pasture larval population. Consequently, chlamydospores are administered orally and deposited in faeces (Ermias *et al.,* 2017)

The endoparasitic fungi are obligate parasite of nematodes, which ingest the parasitic nematodes either by penetration of cuticle from sticky spores adhering to the nematode cuticle and always are of density dependant. *Drechmeria coniospora* is a fungus producing sticky drops on very small conidia, which adhere to the cuticle of the nematode, penetrate the cuticle and destroy the victim. Another endoparasitic fungus, *Harposporium anguillulae* produce very small, half moon shaped conidia which lodge in the digestive tract of the feeding nematode and after germination totally digest the victim before finally breaking through the cuticle to produce new conidia on the short conidiophores. A dose of 3 lakhs conidia/gm faeces could significantly reduce the number of *H. contortus* larvae recovery as per recent study (Chamuah *et al.,* 2013).

The egg-parasitic fungi generally attacked that nematode which has long survival time in outside environment of the host (*Ascaris* spp., *Fasciola* spp., *Amphistomes* spp.). The fungus of *Verticillium chlamydosporium*, other *Verticillium* spp. has also shown the ability to degrade the parasitic egg shell enzymatically and could subsequently infect the eggs. It was also well documented that short exposure to high temperature or UV irradiation rendered the parasitic eggs more susceptible to fungal attack. It has also been shown that *V. chlamydosporium* fungi could attack and destroy the eggs of *Ascaridia galli* and *Parascaris equorum* (Dackman *et al*.,1992; Chamuah *et al.,* 2013)

Pochonia is also one of the most studied genera for its ovicidal activity against helminths that are potentially harmful to agriculture (Braga *et al.,* 2008a). Pochonia chlamydosporia was first found parasitizing eggs and females of Meloydogine sp., in Alabama, United States, 1981. It is considered one of the most promising agents for the management and control of problems caused by phytonematodes. It has also been successfully used to reduce the hatching rate of Ascaris lumbricoides eggs (Hidalgo *et al.,* 2000; Braga *et al.,* 2007). This species is a Deuteromycete, optional parasite of eggs of nematodes that form cysts in roots and branches, with wide distribution (Araujo, 2009).

Table 4: Control measures used in control of parasites with special reference to animal parasites.

Chemical	Non-chemical
Chemotherapy	Biological control
Spraying of poison	Vaccines
Pheromones in insect traps	Selection for host resistance
Insect repellants	Management systems
	Interspecific competition
	Sterile male technique

Source: (Gronvold *et al.,* 1996)

Table 5: Common fungal species used to control nematode parasites

Fungal species		Susceptible nematodes
Endoparasitic fungi	Drechmeria Harposporium anguillucal	Heamoncus species and trichostrongylus colubriformis
Predacious fungi	Arthrobotry soligospora, Arthrobotry misinformis, Arthrobotry robusta, Monacrosporium endermatum	Ostertagia species Dictyocalus villilpar Heamoncus contortus Oesophagostumum species Cooperia Species
Egg parasitic fungi	Vertisillium chlamydosporium	Ascaris suum

Source: (Ermias *et al.,* 2017)

6. Conclusion And Recommendation

Nmatophagus fungi are organisms that can prey, parasitize and digest nematode as source of nutrients this habit of the fungus can make it possible candidate for the use of nematophagus fungus as biological control of nematodes in livestock. Biological control of nematodes becomes an important, integrated element and sustainable strategies to control nematodes in livestock where they can reduce the number of infective nematode eggs in the pasture and adult parasite in the gastrointestinal tracts of infected animals despite of an increase in the resistance ability of nematode to the available anthelmintic drugs. Therefore based on the above conclusive ideas the following recommendations are forwarded:

❖ Production and commercialization of the agent as a treatment option should be promoted.

❖ Further experimental study should be conducted on their efficacy and effects comparing with the available anthelmintic drugs.

❖ Further study should be conducted on the molecular characterization of nematophagus fungi to clearly show the genes responsible for nematocidal activity.

Acknowledgements:

My grateful thank goes to all of the individuals helping me during writing of this review paper.

Corresponding Author:
Dr. Tolessa Ebissa and Dr. Dawit Hora
Department of Veterinary Medicine
Asossa University, Asossa 35099, Ethiopia
Telephone: +251921889089
E-mail: kuletole@gmail.com

Reference

1. Andersson K.M. (2013). Identification of Virulence Factors in Nematode-Trapping Fungi Insights from Genomics, Transcriptomics and Proteomics. Department of Biology, Lund University.
2. Araujo J.M., Araujo J.V., Braga F.R., Carvalho R.O. and Ferreira S.R. (2009): Activity of the nematophagous fungi Pochonia chlamydosporia, Duddingtonia flagrans and Monacrosporium thaumasium on egg capsules of Dipylidium caninum. *Veterinary Parasitology,* 166:86–89.
3. Braga F.R. and Araújo, J.V. (2014): Nematophagous fungi for biological control of gastrointestinal nematodes in domestic animals. *Applied Microbiology and Biotechnology,* 98:71-82.
4. Braga F.R., Araujo J.V., Campos A.K., Carvalho R.O., Silva A.R., Tavela A.O. and Maciel A.S. (2007): Observac¸a˜o in vitro da ac¸a˜o dos isolados fu´ ngicos Duddingtonia flagrans, Monacrosporium thaumasium e Verticillium chlamydosporium sobre ovos de Ascaris lumbricoides (Lineu, 1758). *Revista da Sociedade Brasileria de Medicina Tropical*, 40:356–358.
5. Braga F.R., Araujo J.V., Campos A.K., Silva A.R., Araujo J.M., Carvalho R.O. and Tavela A.O., (2008a): In vitro evaluation of the action of the nematophagous fungi Duddingtonia flagrans, Monacrosporium sinense and Pochonia chlamydosporia on Fasciola hepatica eggs. *World Journal of Microbiology and Biotechnology,* 24:1559–1564.
6. Chamuah J.K., Perumal P. and Mech A. (2013): Epidemiology and Control Measures of Helminth Parasites in Small Ruminants *International Journal of Bio-resource and Stress Management,* 4(2):278-287.
7. Chen S. and Dickinson D.W. (2004): Biological control of nematodes by fungal antagonists. In: Chen, Z., Chen, S., Dickinson, D.W. (Eds.), Nematology Advances and Perspectives, vol. II. Nematode Management and Utilization. Tsinghua University Press & CABI Publishing, Beijing & Wallingford, Pp. 979–1039.
8. Dackman C., Jansson H-B. and Nordbring-Hertz B. (1992): Nematophagus Fungi and their activity in soil, Lund University, Lund, Sweden.
9. De S. and Sanyal P. K. (2009): Biological Control of Helminth Parasites by Predatory Fungi. *www.vetscan.co.in.*
10. Degenkolb T. and Vilcinskas A. (2016): Metabolites from nematophagous fungi and nematicidal natural products from fungi as an alternative for biological control. Part I: metabolites from nematophagous ascomycetes. *Applied Microbiology and Biotechnology*, 100:3799-3812.
11. Dijksterhuis J., Veenhuis M., Harder W. and Nordbring-Hertz B. (1994): Nematophagous fungi: physiological aspects and structure-function relationships. *Advanced Microbiology and Physiology*, 36:111-143.
12. Epe C., Holst C., Koopmann R., Schnieder T., Larsen M. and Von Samson-Himmelstjerna, G. (2009): Experiences with Duddingtonia flagrans administration to parasitized small ruminants. *Veterinary Parasitology,* 159:86-90.
13. Ermias W., Ashenafi K., Hagos A. and Biniam T. (2017): Alternative Control Methods of Gastrointestinal Nematode Infections in Small Ruminants: Biological Method and Use of Medicinal Plant Extracts. *ARC Journal of Animal and Veterinary Sciences*, 3 (2) 11-27.
14. Falzon L.C., van Leeuwen J., Menzies P.I., Shakya K.P., Jones-Bitton A. and Sears W. (2013): Anthelmintic resistance in sheep flocks in Ontario. *Canada Veterinary Parasitology*, 193:150–162.
15. Filippe E. F. S., Bruna L. S. and Jose H. Q. (2018): Nematophagous fungi: Far beyond the endoparasite, predator and ovicidal groups. *Agriculture and Natural Resources,* 52:1-8.
16. Gray N. F. and Smith R. I. L. (1984): The distribution of nematophagous fungi in the maritime Antarctic. *Mycopathologia*, 85: 81-92.
17. Gronvold J., Henriksen S.A., Larsen M., Nansen P. and Wolstrup J. (1996): Biological control: Aspects of biological control, with special reference to arthropods, protozoans and helminthes of domesticated animals. *Veterinary Parasitology,* 64:47-64.
18. Hidalgo L., Bourne J.M., Kerry B.R. and Rodrıguez-Kabana M.G. (2000): Nematophagous *Verticilium spp*. in soils infested with *Meloidogyne spp.* in Cuba: isolated and screening. *International Journal of Pest Management*, 46:277–284.

19. Hsueh Y.P., Mahanti P., Schroeder F.C. and Sternberg P.W. (2013): Nematode-trapping fungi eavesdrop on nematode pheromones. *Current Biology,* 23:83-86.
20. Jansson H.B., Persson C. and Odselius R. (2000): Growth and capture activities of nematophagous fungi in soil visualized by low temperature scanning electron microscopy. *Mycologia,* 92:10–15.
21. Jansson H.B., Tunlid A. and Nordbring-Hertz B. (2006): Nematophagous Fungi. *Encyclopedia of Life Sciences*. Pp 1-11. doi: 10.1038/npg.els.0004293.
22. Larsen M. (2000). Prospects for controlling animal parasitic nematodes by predacious micro fungi. *Parasitology*, 120:121-131.
23. Larsen M. (2006). Biological control of nematode parasites in sheep. *Journal of Animal Science,* 84:133-139.
24. Li J., Zou C., Xu J., Ji X., Niu X., Yang J., Huang X. and Zhang K.Q. (2015): Molecular mechanisms of nematode-nematophagous microbe interactions, basis for biological control of plant-parasitic nematodes. *Annual Review on Phytopathology*, 53:67-95.
25. Liang L., Wu H., Liu Z., Shen R., Gao H., Yang J. and Zhang K. (2013): Proteomic and transcriptional analyses of *Arthrobotrys oligospora* cell wall related proteins reveal complexity of fungal virulence against nematodes. *Applied Microbiology and Biotechnology*, DOI 10.1007/s00253-013-5178-1.
26. Liu W., Han Y., Wang B., Sun L., Chen M., Cai K., Li X., Zhao M., Xu C., Xu Q., Yi L., Wang H., Xie D., Li X., Wu J., Yang J., Wei S., Li D., Chen C., Zheng T., Li Q. and Peng J. (2015): Isolation, identification, and characterization of the nematophagous fungus Monacrosporium salinum from China. *Journal of Basic Microbiology*, 54:1–10.
27. Liu X., Xiang M. and Che Y. (2009): The living strategy of nematophagous fungi. *Mycoscience*, 50:20–25.
28. Lopez-Llorca L.V., Macia-Vicente J.G. and Jansson H.B. (2008): Mode of Action and Interactions of Nematophagous Fungi. *Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes*, Pp. 51–76.
29. Maciel A.S., Araujo J.V., Campos A.K., Lopes E.A. and Freitas L.G. (2009): Predation of Ancylostoma spp. dog infective larvae by nematophagous fungi in different conidial concentrations. *Veterinary Parasitology,* 161:239–247.
30. Masoomeh S.G., Mehdi R.A., Shahrokh R.B., Ali E., Rasoul Z. and Majid E. (2004): Screening of Soil and Sheep Faecal Samples for Predacious Fungi: Isolation and Characterization of the Nematode-Trapping Fungus Arthrobotrys oligospora. *Iranian Biomedical Journal*, 8 (3):135-142.
31. Mota M.A., Campos A.K. and Araujo J.V. (2003): Controle biolo´ gico de helmintos parasitos de animais: esta´ dio atual e perspectivas futuras. *Pesquisa Veterinaria Brasileria,* 23:93–100.
32. Nordbring-Hertz B., Jansson H.B. and Tunlid A. (2006): Nematophagous Fungi. In: *Encyclopedia of Life Sciences*. John Wiley & Sons, Ltd, Chichester, Pp. 1-11.
33. Oliveira I.C., Carvalho L.M., Vieira I.S., Camposa A.K., Freitas S.G., Araujo J.M., Braga F.R. and Araújo J.V. (2018a): Using the fungus *Arthrobotrys cladodes* var. *macroides* as a sustainable strategy to reduce numbers of infective larvae of bovine gastrointestinal parasitic nematódeos. *Journal of Invertebrate Pathology,* 158:46-51.
34. Persmark L., Banck A. and Jansson H.B. (1996): Population dynamics of nematophagous fungi and nematodes in an arable soil: vertical and seasonal fluctuations. *Soil Biology and Biochemistry journal*, 28:1005-1014.
35. Sargison N.D. (2012). Pharmaceutical treatments of gastrointestinal nematode infections of sheep future of anthelmintic drugs. *Veterinary Parasitology*, 189:79–84.
36. Saumella C.A., Fernándezb A.S., Fuséa L.A., Rodrígueza M., Sagüésa M.F. and Lucía Emilia Iglesias L.E. (2015): Nematophagous fungi from decomposing cattle faeces in Argentina. *Revista Iberoamericana de Micologia,* 32(4):252–256.
37. Vieiraa I.S., Oliveiraa I.C., Camposa A.K. and Araújoa J.V. (2019): Association and Predatory Capacity of fungi *Pochonia chlamydosporia* and *Arthrobotrys cladodes* in the biological control of parasitic helminths of bovines. Available at https://www.cambridge.org/core/terms /doi.org/10.1017/S003118201900060X.
38. Von Reuss S.H., Bose N., Srinivasan J., Yim J.J., Judkins J.C., Sternberg P.W. and Schroeder F.C. (2012): Comparative metabolomics reveals biogenesis of ascarosides, a modular library of small-molecule signals in *C. elegans*. *Journal of American Chemical Society*, 134:1817-1824.
39. Waller P.J. (2006). Sustainable nematode parasite control strategies for ruminant livestock by grazing management and biological control. *Animal Feed Science and Technology*, 126:277-289.

40. Waller P.J. and Faedo M. (1996): The prospects for biological control of the free-living stages of nematode parasites of livestock. *International Journal of Parasitology,* 26:915-925.
41. Ya-Juan X., Er-Le L., Cai-Xia J., Li M. and Kui-Zheng C. (2018): Isolation, identification and characterization of the nematophagous fungus *Arthrobotrys* (*Monacrosporium*) *sinense* from China. *Acta parasitological,* 63(2):325–332.
42. Yang E.C., Xu L.L., Yang Y., Zhang X.Y., Xiang M.C., Wang C.S., An Z.Q. and Liu X.Z. (2012): Origin and evolution of carnivorism in the Ascomycota (fungi). *Proceedings of the National Academy of Sciences of the United States of America,* 109:10960-10965.
43. Zare R., Gams W. and Evans H. C. (2001): A revision of *Verticillium* section *Prostrata*. V. The genus *Pochonia*, with notes on *Rotiferophthora. Nova Hedwigia,* 73:51–86.

8/3/2019