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Overview of nematophagous fungi, isolation techniques, and their role in biological control of helminthic parasites: A literature review

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Abstract
Nematophagous fungi are natural predators of soil-dwelling nematodes and can detect and respond to their prey's ascarosides pheromones. They can be endoparasitic, nematode-trapping, egg-parasitic, or toxin-producing depending on how they attack worms. They are found throughout the world in a wide range of habitats and climates, but few are from extreme environments. They are mostly concentrated in the upper part of the soil, in pastures, leaf litter, mangroves, and certain shallow aquatic habitats. They use methods including non-constricting loops, adhesive hyphal strands, adhesive knobs, adhesive nets made of hyphal threads, and hyphae loops that tighten around caught worms. There are numerous advantages for the ecology and economy that come from nematophagous fungi in the soil. They help promote the cycling of nutrients and stabilize soil ecosystems. They are also used to protect plants and animals from nematode disease and avoid drug resistance. However, there is no comprehensive review assessing the above roles and, therefore, this review intended to assess the general overview of nematophagous fungi, their agroecology, isolation and identification, and their role in biological control of helminthic parasites.

Keywords: Nematophagous fungi, nematode, biological control

Introduction
Fungi are pathogenic, parasitic, or symbiotic with a range of different animals, but their relationship with soil nematodes goes a step beyond parasitism and into predation [1]. Nematodes belong to the phylum Nematoda, which has digestive, nervous, secretory, and reproductive systems, but does not have a circulatory or respiratory system [2].
Microfungi that can capture, kill, and eat nematodes are nematophagous fungi [3-5]. They are natural predators of soil-dwelling nematodes and can detect and respond to their prey's ascaroside pheromones [6]. They capture vermiform worms using unique mycelial structures known as "traps", or they target nematode eggs and cysts with hyphal tips before the nematode cuticle is penetrated, invaded, and digested. Their saprophytic and parasitic abilities vary. While many egg-parasitic and trap-forming fungi can grow saprophytically in soil, endoparasites mostly rely on nematodes for nutrition [7]. These fungi can be found in soil and other organic substrates, particularly in dung [8]. Although nematophagous fungi often like organic soils, their low dietary and vitamin requirements allow them to proliferate in almost any kind of soil [9]. As biological control agents, they are crucial in animal husbandry and agriculture [10].
Nematophagous fungi use several methods to hunt their prey. These methods include living within the nematode and slowly consuming them as well as spreading diseases through nematode populations. They are responsible for keeping the nematode population in check and are an important part of the subsoil ecosystem [11]. A large and varied number of different fungi that feed on nematodes are known as nematophagous fungi. They fall into four groups: nematode-trapping fungi (NTF), endoparasitic fungi, fungi that parasitize females and eggs, and fungi that produce toxins [7]. Being obligate parasites, the endoparasites rely solely on nematodes for nourishment. They infect nematodes with either adhesive or non-adhesive spores. The NTF relies on their capacity to eat nematodes to varying degrees given that they can also feed saprophytically [12].

In grazing animals, intestinal nematodes result in significant productivity losses and are a global issue for animal welfare. Anthelmintics have been a mainstay of these parasite control efforts for a long time. Due to the excessive usage of anthelmintic medications, there are now issues with resistance to all classes of current broad-spectrum anthelmintics, including benzimidazoles, imidothiazoles-tetrahydropyrines, and macrocyclic lactones [13]. As time has passed, problems of multi-resistance to more than one class have occurred as well. Multi-resistant nematodes have become a major threat to the whole small ruminant industry [14]. Every naturally occurring grazing species in our range, including sheep, goats, cattle, and horses, has colonies of resistant nematodes [13]. The need for the development of biological control agents in crop protection has significantly increased in the modern era due to worries about chemical nematicides and their effects on the environment and human health. The main factor driving the increased interest in nematophagous fungi is their potential as bio-control agents against nematodes that parasitize plants and animals [15]. Therefore, this review aimed to provide an overview of nematophagous fungi and their role in the biological control of helminthic parasites.

This review has the following sections: 1) general overview of nematophagous fungi, 2) their agroecology, 3) virulence factors, 4) isolation and identification, and 5) their role in biological control of helminthic parasites.

Overview of Nematophagous Fungi

Nematophagous fungi are cosmopolitan microorganisms capable of regulating their saprophytic conduct to carnivorous, permitting them to feed on nematodes below unfavorable dietary conditions. Over 700 different phyla, including the Ascomycota, Basidiomycota, Chytridiomycota, Zygomycota, and Oomycota, are known to have nematophagous fungus species [16]. They have extremely complex infection techniques and are nematodes' natural adversaries [17, 18]. Nematophagous fungi are those that have evolved to use nematodes as their primary food source. These fungi can be further classified into two groups: predatory fungi (also known as nematode-trapping fungi) that use specialized hyphal devices to catch their prey, and endoparasitic fungi that produce spores that infect nematodes. One of the fungal life's tactics for eliminating nematodes is predation [19]. Their specialty is ensnaring and breaking down nematodes. Certain species remain within the nematodes from the beginning, while others catch them using glue traps or rings, some of which contract upon contact. Certain species have both kinds of traps. Another approach used by Stropharia rugosoannulata, Coprinus comatus, and the Pleurotaceae family is to stun the nematodes with poisons [20]. Nematophagous fungi are predators, therefore their ability to feed may be restricted. This means that its population is constantly in check by its prey. Additionally, it controls the nematode population. These nematodes are also innumerable worldwide which results in a sTable supply for soil ecosystems [21].

Based on how they attack nematodes, nematode-trapping fungi (predacious/predatory fungi), endoparasitic fungi, parasitic fungi that feed on eggs and female worms, and fungi that produce toxins are the four categories into which nematophagous fungi are classified (Table 2). Nematophagous fungi have been shown to synthesize extracellular enzymes, which may play a role in their parasitism [22].

Nematode-Trapping Fungi

NTF are soil-borne fungi that use a variety of shaped and sized trapping structures to capture the nematode life cycle as it moves. These fungi attract all nematodes that live in the soil and are not host-specific. Trapping devices have the potential to form spontaneously or in response to nematodes or proteinaceous substances [23]. Adhesive or mechanical hyphal traps are used by NTF. Adhesive networks, adhesive hyphae, constricting rings, adhesive knobs, and non-constricting rings are the five types of trapping devices that have been identified [9].

On surfaces coated in an adhesive coating, an adhesive hyphae short, erect branch with a few swollen cells on it-forms. The most prevalent kind of traps are the sticky networks. They seem like a web of interconnected loops growing out of the ground. A globose or sub-globose cell with an erect stalk or that is sessile on the hypha is known as the sticky knob [24]. Three cells that are constant in size and shape make up the non-constricting rings. They are always found next to sticky knobs. The nematode's fate is set after attachment. Firmly connected to the nematode, the fungal trapping organ will eventually break free of the hyphae, staying attached to the nematode and starting the infection process, even if it struggles to do so. The most complex trapping mechanism, constricting rings have three rings and are frequently found in the species *Drechslerella anchonia* (*Arthrobotrys*). In an attempt to obtain nourishment, the worm wriggles inside the ring, but as soon as it touches it, something happens [25].

Different fungal species develop different kinds of trapping devices. These structures range in complexity from very basic fungal hyphae coated in sticky secretions to considerably more intricate ones. They can be two or three-dimensional networks, simple loops, adhesive branches (*Arthrobotrys oligospora*), or any combination of these. The most common type of fungal traps are adhesive three-dimensional nets, which are constructed as the loops form a three-dimensional arrangement. Adhesive spores (*Meristacrum* spp.) or adhesive knobs (*Arthrobotrys haptotyla*, *Nematoctonus spp*., and *Gamsylella robusta*) are produced by other species of trapping fungi. Vegetative hyphae lateral branches form non-constricting rings that wedging around the nematodes' bodies ensnaring them (*Dactylella leptospora*) [26]. The most specialized traps are constrictin rings (*Arthrobotrys dactyloides*, *Monacrosporium doedycoides* and *Drechslerella stenobrocha*), which have three cells that swiftly inflate to grip the worm firmly as it enters [7, 27].

Even within a genus, trapping structures can vary. For instance, *Nematoctonus robustus* only produces sticky knobs on hyphae, *Nematoctonus leptosporus* only on germinated conidia, and *Nematoctonus angustatus* on both hyphae and conidia. Their potential for biological control is diminished by a few drawbacks, including their non-specific trap of plant-parasitic nematodes, limited catching activity, and complexity in soil establishment [27]. It has been demonstrated that ensnaring fungi can also release antibacterial and nematicidal substances such as pleurotin (N. Robustus, N. Concurrens) or linoleic acid (A. Oligospora, A. Conoides). There was a positive link between the amount of traps produced and the production of linoleic acid. The number and population densities of

nematode-trapping species found in a particular soil can vary significantly. Typically, the upper 30 cm of soil and the fall have the highest concentrations [9].

Endoparasitic Fungi

They don't hunt using hyphae [17]. Nematodes are their primary food source. Although the majority of those fungi are obligate parasites with horrible saprotrophic competition in soil, they often have a large host range for nematodes. These obligatory parasites remain within their afflicted hosts throughout their whole vegetative life cycle [7, 27]. Through their spores, endoparasitic fungi infect vermiform nematodes (conidia or zoospores). The nematode can contract the spores in two different ways: (i) pre-oral, in which the spores enter the mouth through food that the nematodes eat together, or (ii) percutaneous, in which the spores stick to the nematode's cuticle. The spores can be consumed by the nematode, which either clings tightly to its cuticle while passing the fungus, or germinates inside the intestines (usually the esophagus or mastax). With what appears to be some mechanical pressure, the spore contents are injected into the nematode via a thin penetration tube. Next, an internal mycelium develops and eventually makes its way through the cadaver's surface to sporulate [28]. Zoospores produced by certain endoparasitic fungus swim in the direction of the worm, adhere to the cuticle typically around the natural openings, and then encyst. Through the person's herbal apertures, the encysted zoospores enter the host frame and start their vegetative growth. The hyphae then produce some sporangium that contains zoospores [27]. There is endoparasitic activity in *Nematoctonus concurrent* and *Nematoctonus haptocladus* [16].

Egg/Female Parasitic Fungi

Along with the various nematodes that migrate, some plant-pathogenic nematodes also spend the majority of their life cycle inside plant roots, either in cysts or on the floor in root knots. These inactive levels remain in the soil and serve as a favorable substrate for the colonization of fungi by egg parasites. Along with *Meloidogyne spp*. and *Tylenchulus semipenetrans*, numerous opportunistic soil fungus had been extracted from the eggs, cysts, and sedentary ladies that deposit their eggs in gelatinous matrices [10, 27].

Fungi that parasitize eggs and cysts are more common than those that infect females. Appressoria or zoospores are the means by which this group of fungi infects their hosts [7]. The great potential of egg and sedentary stage parasites in the biological control of economically significant nematodes has garnered more attention. Because their host is sessile (eggs, developing juveniles, and females), these fungi are more effective at spreading because they may thrive saprotrophically in the rhizosphere and are relatively simple to mass-culture. Relatively few nematode parasitizing fungus have been identified as potential bio-control agents [29].

Toxin-Producing Fungi

Before hyphae may pass through the worm cuticle, the toxin secreted by the toxin-producing fungi renders the nematodes immobile. *In vitro*, nematophagous fungus released a number of substances that might be nematostatic or nematocidal. Pleurotus ostreatus secretes droplets of a strong toxin with the structure of trans-2-decenedioic acid that rapidly immobilizes nematodes, while the *in vivo* function of such compounds is typically unknown. *Pleurotus ostreatus* and *Coprinus comatus* are two examples of the species of the *basidiomycetous*, *Pleurotus*, and *Coprinus* genera that have been shown to produce toxins [30]. It's possible that more Basidiomycota members practice nematophagy than previously believed [7]. *Drechmeria coniospora*, *Harposporium anguillulae* [9], *Lecanicillium*, and *Paecilomyces lilacinus* have all been shown to have antibiotic (nematicidal and antifungal) activity. Additionally, *Paecilomyces lilacinus* secretes acetic acid, which paralyzes young nematodes [31].

Ecology and habitat

Few nematophagous mushrooms are found in harsh conditions, although they are found worldwide in a variety of habitats and climates [32-33]. There are many species that are unrecorded, but the majority of studies have explained that they are nematophagous species that attack the nematodes of hobby farmers, horticulturists, and foresters. Orbilia's asexual stage is found in freshwater, marine, and terrestrial environments, whereas its sexual stage is found on decaying wood on land or in freshwater. Other species have been reported on mangroves, but *Arthrobotrys dactyloides* was the first to be found in brackish water [33].

Nematode-trapping fungi are primarily found in the upper soil, in mangroves, pastures, leaf litter, and certain shallow aquatic environments. They use methods including non-constricting loops, adhesive hyphal strands, adhesive knobs, adhesive nets made of hyphal threads, and hyphae loops that tighten around any caught worms. After the worm is confined, its internal tissues are consumed by the hyphae after they break through the cuticle [34].

Arthrobotrys oligospora has been reported from Asia, Africa, North and South America, Australia, and other continents. *Arthrobotrys oligospora* was more prevalent when nematode-infected insects were present, but not other nematode-capturing fungi. The fungus is present in the soil of plantations, grasslands, shrublands, sheep and cow yards, and in the excrement of both domesticated and wild animals [12]. In general, several soil variables-such as pH, moisture, nutrients (N, P, and K), heavy metals, and nematode density-are linked to the distribution and abundance of nematode-trapping species and groups of fungus [32, 35]. Woodland steppe soil, mixed woodland soil, and Mediterranean brown soil (pH 6.9-8.0), where the pH can drop as low as 4.5 but is typically higher than 5.5, are among the soil types it colonizes. The fungus has also been detected in aquatic habitats and highly contaminated places, particularly in mines that have leached heavy metals, in soil that has been infected with fungicides or nematicides, decomposing plant matter, leaves, roots, and moss, as well as in the rhizosphere of different bean plants, barley, and tomato plants [12]. Late spring and summer are when the fungus is most prevalent [31].

It has been found that the fungus *Arthrobotrys oligospora*, which makes nets, can detect the presence of nearby nematodes in the soil and can best prepare its snares during their presence. This is most likely because creating the internet requires an enormous amount of energy; the fungus detected the pheromones, such as ascarosides, that the worms use to communicate, alerting it to the nematode's presence. The fungus actively seeks out its prey by producing scent cues that resemble those used by the computer virus to find food and attract partners. A hypha's

loop is used by the species *Arthrobotrys dactyloides* to capture nematodes. When a nematode tries to escape via the loop, the loop constricts extremely quickly, trapping the worm [36].

Virulence factors involved in infection of Nematophagous fungi

Serine protease, chitinase, and collagenase are examples of extracellular enzymes that are engaged in the infectious process as virulence factors. These enzymes correspond to the primary chemical components of the nematode cuticle and eggshell [37].

Chitinases

They are a crucial component of invertebrate cuticles, accelerating the synthesis of chitin, and are necessary for hyphal development. They also play a role in the infection of mycoparasites and entomopathogenic or nematopathogenic fungi. The possible function of fungal chitinase in nematode egg infection. The most prevalent amino polysaccharide in nature, chitin, is a stiff and resilient structural element that enhances the mechanical strength of organisms that contain it. It is essentially a linear cationic heteropolysaccharide made up of D and N-acetyl-D-glucosamine units. A chitinolytic device that functions in a synergistic and sequential manner is used to carry out the enzymatic breakdown of chitin. Numerous species, whether or not they contain chitin, generate an amazing class of chitinolytic enzymes with unique catalytic characteristics and specificities. Their physiological functions include defense, morphogenesis, chitin recycling, feeding, and/or parasitism [38].

Since microorganisms are the main degraders of chitin in the environment, they are a vital natural source of chitinolytic enzymes. The biological control system naturally arises from the interactions between various organisms. Because chitinolytic enzymes may be involved in hostility toward pathogenic chitin-containing organisms, there has been a surge in interest in this field within organic management. Since plants and vertebrate animals do not contain chitin, safe and specific "target" molecules can be considered for the management of pathogenic microbes harboring chitin. Because they produce enzymes that have an antagonistic influence on insects, fungi, and nematodes, fungi exhibit suiTable properties as possible biological control agents [39].

Collagenases

The three-layered, fibrous ascarid cuticle is made up of certain keratin and collagen types found in nematodes. One of the most complicated types of proteins, collagens break down gradually in natural soils and streams. Nematophagous fungi need to break through the worm cuticle in order to infect nematodes. Collagenases in particular have been identified as critical enzymes in the pathogenicity of nematophagous fungi [40, 41].

Subtilisin-like serine protease (SLSP)

A subfamily of enzymes known as SLSPs breaks down protein substrates. SLSPs can function as pathogenicity or virulence factors in fungi, or they can have particular roles in cell metabolism or general nutrition [42]. Aspartic acid, histidine, and serine make up the catalytic domain of SLSP, a virulence enzyme, and its substrate has a serine residue that is reactive with organic phosphate fluorine. Prokaryotic and eukaryotic organisms have subtilisin-like proteases, which are a large class of serine proteases with a variety of roles, including the special processing of various proproteins and prohormones. The penetration and digestion of worm cuticles are facilitated by subtilisin-like serine proteases found in nematode-trapping fungi [43].

Lectins

It has long been believed that the interaction between lectins on the surface of trapping devices or sticky spores and carbohydrate ligands at the nematode cuticle may be what mediates the nematophagous fungi's adherence to their host. All living things include lectins, which are proteins that bind carbohydrates. The genomic inventory of nematode pathogenic and insect pathogenic fungi was compared to that of trap-forming fungus, and the results showed that the former had significantly more lectin-encoding genes than the latter. All entice-forming fungi produced transcripts encoding RicinB-lectins during entice creation and infection; these transcripts may be ribosome-inactivating proteins (RIPs) combined with a catalytic A-chain and a sugar-binding B-chain [44].

Secondary Metabolites

In addition to being involved in the relationship between fungi and the pests they inhabit, they are also the starting point for the development of medications, insecticides, and nematodes [45]. Many microbes develop harmful compounds like antibiotics to fend off or even kill their rivals. Because they weaken the host and make infection easier, toxins are especially crucial for parasitic microbes [14]. Most recognized nematicidal secondary metabolites are those that are generated in collaboration with opportunistic fungus. Compounds produced by Fusarium equiseti slow down the hatching of egg-knot nematode worms and render infectious juveniles immobile. These metabolites are crucial to the endoparasite's ability to destroy the host. Because of their ability to oppose phytopathogenic fungi, *Trichoderma spp*. are biocontrol agents that are frequently employed in plant protection [46].

Nematode-Fungus Interaction Mechanism

Both nematodes and nematophagous fungi exhibit a unique predator-prey dynamic. Like many other soil dwellers, the nematodes release compounds into the soil as they move. These nematodes' prey, fungi, have developed the ability to recognize and react to their presence. Additionally, the fungi have created a variety of intricate strategies for ensnaring the nematodes by attacking them from the exterior as well as the inside [16]. Numerous fungal species have evolved the capacity to identify certain compounds that worms utilize for communication and growth, enabling them to pursue their prey with efficiency. When the nematodes stray too close to the fungus, several chemical substances are found. The fungus will set up traps in the vicinity where it detects the compounds if it needs nutrients that are not present in its diet, effectively hunting its prey [47].

A relatively conserved circle of tiny compounds called ascarosides is produced by nematodes. The fungus are able to identify these tiny chemicals, which are specific to nematodes. Various nematode species create different forms of ascarosides. When these ascarosides are discovered, nematode ensnaring traps are constructed. The fungus in

issue behaves reactively as opposed to being proactive and expending valuable energy to build trap structures that might never be used. Inside the area where the ascarosides were found, traps are constructed. But this is not the usual behavior. A select few fungal species, including *Arthrobotrys oligospora*, are capable of this chemical eavesdropping phenomenon, and they only do so when they are malnourished. Because these constructions require a lot of energy, the fungus must be hungry and aware of its prey's location before it will begin to set traps [6]. Certain nematophagous fungi release toxins that render nematodes immobile. For instance, the Coprinus comatu hypha uses a structure called a spiny ball to attack the free-living soil nematode Panagrellus redivivus. This allows the hypha to enter the skin and consume the contents of the nematode after damaging its cuticle to cause immobilization [30].

Spores from endoparasitic fungi are drawn to soil nematodes and tend to gather in the tissues that the nematodes are absorbing. From there, escape tubes grow through the cuticle, and eventually, motile spores exit through those as well, ready to infect more worms. In other fungal species, the nematode encounters conidia instead of spores, which infect it in a similar manner [48]. The nematode consumes the sickle-shaped conidia of Harposporium anguillulae and lodges them in the esophagus or gut, where they penetrate the tissues [49].

In species that feed on eggs, the hypha flattens against the egg; the emergence of appressoria signals the impending or current infection. Subsequently, the hypha penetrates the egg and consumes the growing juvenile nematode, subsequently generating conidiophores and proliferating towards adjacent eggs [50]. Nematophagus fungus can create unique attacking tools and employ a variety of tactics in the biological control process [51]. Specialized weapons function similarly to a sharp sword, cutting through the worm cuticle to cause extravasation of the nematodes' internal substance and allowing fungal hyphae to completely colonize the nematode body. In general, the operation of these special gadgets is as follows: In order to infect a nematode, hyphae must first: 1) expand toward and press against its cuticle; 2) produce a penetration peg that pierces the worm's cuticle; 3) inhabit the nematode's interior; and 4) project themselves from the nematode (30). The nematode attraction, adhesion, penetration, and digesting mechanisms, as well as the mechanisms underlying the capture process [11], are responsible for this development.

Recognition

The process by which nematophagous fungus identify their prey is a complicated one. None of the nematode-trapping species have been shown to exhibit simple host specificity, although studies using the endoparasite *Drechmeria coniospora* have demonstrated a slightly higher level of host specificity. However, it seems that at various stages of the fungus-nematode interaction, there are recognition events in the cell-cell communication that could trigger a specific physiological, morphological, or biochemical reaction [4]. Nematodes are drawn to the fungus's mycelia, which have the potential to create traps. They are also drawn to fully grown spores and traps. The use of "quick range" or touch communication is combined with this. The ensuing stages of the infection, such as the nematode cuticle's penetration, most likely depend on the host's recognition [16].

Attraction

Compounds emitted from the mycelium, nematode-trapping fungus' traps, and endoparasite spores all attract nematodes. The form and, thus, the saprophytic/ parasitic properties greatly influence the fungus's appeal. It appears that more parasitic fungi are more attractive than more saprophytic ones; in other words, endoparasitic species that infect worms with conidia are more successful in drawing nematodes than larger saprophytic species that use a variety of trapping mechanisms. Volatile diffusing chemicals and volatile exudatives seemed to be attractive substances.

Adhesion

For fungal parasites to be able to infect, they must adhere to their host. The extracellular matrix (ECM) or fungal sheath mediates adherence in the majority of pathogenic and parasitic fungi [52, 53]. In this stage, a fungus's lectin, a protein that binds carbohydrates, interacts with the nematode's carbohydrate receptor. Under an electron microscope, nematodes can be seen contacting and adhering to the spores and traps of nematophagous fungi. Even before to contact with the nematodes, the extracellular fibrils in *Arthrobotrys oligospora* envelop the three-dimensional nets. Once in contact, these fibrils align themselves perpendicular to the surface of the host, most likely to facilitate the subsequent fungal invasion and attachment of the nematode. Whether or not contact with the nematode has been formed, the endoparasite *Drechmeria coniospora* exhibits an entirely different kind of adhesive that appears to be made of radiating fibrils. Moreover, *Drechmeria coniospora* spores cling specifically to the sensory organs at the apex of the nematode's head, inhibiting nematode attraction. Although the exact chemical makeup of nematophagous fungi's surface filaments is unknown, it is known that they contain both proteins and polymers that contain carbohydrates [54].

Constricting Rings

Even if other predatory fungi's nematode infection patterns employ sticky layer to catch nematodes (nets, hyphae, or knobs). Constricting rings work on a total distinct process of entrapment. The three cells that make up the 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. In addition, various triggers like heat or a needle touching a ring's inner (luminal) surface can cause the trap to collapse. The reaction occurs in 0.1 s, is irreversible, and results in a large rise in cell volume that nearly shuts the trap's aperture. The fungus punctures the nematode cuticle by producing a penetration tube once it has been captured**.** A little infection bulb that gives rise to trophic hyphae develops inside the nematode [55]. 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 defined line on the inner surface of the ring during the process of ring cell enlargement. 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 implicated in the ring cell expansion has been studied [56]**.** In this fungus, it seems that the ring cells' glycoproteins are activated by the pressure that a nematode applies to them. Water channels open as a result of the activation, which also raises the cytoplasmic Ca^{2+} level and activates calmodulin. The ring cells enlarge, narrowing the ring and rendering the

worm immobile [57].

Penetration

The traps' adherence to the worm causes the fungus to differentiate. *A. oligospora* is the source of the penetration tube that pierces the worm cuticle. 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. Most of the proteins in the nematode cuticle, including collagen, are present. Numerous proteases from nematophagous fungi have been found to hydrolyze cuticle proteins. These proteases are all members of the serine protease family, and research using sequencing data has shown a strong resemblance between them and the serine protease subtilisin [4]. A chymotrypsin-like protease appears to be involved in the penetration process of the endoparasite *Drechmeria coniospora*. In addition to its involvement in the entry and digestion of the cuticle and tissues of infected nematodes, more thorough investigations of the subtilisin PII generated by *A. oligospora* have revealed that this type of protease can perform multiple distinct activities, including apparent neurotoxic action [52].

Nutrient digestion and storage

After penetrating, the nematode is digested by the fungus that causes infection. After entering the nematode, *A. oligospora's* penetration tube expands to create a sizable infection bulb. The trophic hyphae and bulb development occur concurrently with notable modifications to the fungus's ultrastructure and physiology. The trap cells and the bulb break down the dense bodies. Normal cell organelles are usually seen in the bulb and the trophic hyphae, with the endoplasmic reticulum being especially well-developed. Subsequently, lipid droplets build up in the trophic hyphae, which are most likely used for storing and assimilating nutrients from the infected worm. The endoparasite *Drechmeria coniospora* differs from the trap-forming fungus in that it does not contain thick bodies, which are characteristic of the trap-forming fungi and does not create an infection bulb upon penetration. A. oligospora can also store nutrients from the host by producing a lot of lectin in the cytoplasm in addition to forming lipid droplets. This protein, known as *Arthrobotrys oligospora* lectin, or AOL, belongs to a unique family of low molecular weight lectins that have only been found in a few filamentous fungi to yet. The family shares comparable basic sequences and binding characteristics [4]. After worms have been ingested and digestion has begun, A. oligospora quickly synthesizes AOL during the nematode infection. AOL builds up in large quantities inside the trophic hyphae of the nematode. Later, the lectin is moved from the worm that is infected to other areas of the mycelium, where it can break down and aid in the fungus's growth. It has been proposed that AOL, like other lectins, participates in a recognition event during the interaction with the nematodes, even if the exact processes are unknown. This theory is supported by the observation that the AOL family of lectins binds to sugar structures that are typical of animal glycoproteins, such as worms, but not of fungi [40].

Isolation and identification of nematophagous fungi

Culture Media For-Fungi

Nematophagous fungi have been isolated and incubated using a variety of growth conditions. Generally speaking, though, isolation requires the use of low-nutrient media while incubation requires the use of high-nutrient media. Nematophagous fungi are widely cultured in PDA, WA, CMA, Oat meal medium, Maize meal agar, Rabbit-dung agar [58], Difco CMA [59] and Selective media [60] once they have been isolated. It should be mentioned, though, that nematophagous fungus will often produce fewer conidiophores in the presence of greater resources [61].

Isolation techniques

Nematophagous fungi can be isolated using a variety of methods, including differential centrifugation, the Baermann funnel technique, and soil sprinkling [3]. These methods are efficient and quick ways to get a general idea of the variety of nematophagous fungi that infect living nematodes in soils. Quantitative analyses can also be conducted. For measuring nematode-trapping fungi, the soil dilution approach and soil sprinkling technique work well [62], whereas the differential centrifugation technique works well for quantifying endoparasitic fungi. In the soil dilution and soil sprinkling approaches, nematodes are employed as baits to enable microscopic observation of the fungi. Limitations include labor and time-intensiveness as well as the inability to determine if the fungus are present in the soil as hyphae or spores. Endoparasitic organisms are primarily isolated using the Baermann funnel technique and differential centrifugation technique [63].

Soil Sprinkling Technique

The first nematode-trapping fungus were identified by [58], plating tiny quantities of soil onto nutrient agar. But the saprobic fungi outperformed the nematode-trapping fungus, growing faster and swiftly colonizing plates. In order to select for nematode-trapping fungi, [64] added nematodes to the nutrient agar, converting it to 2% water agar. The low nutritional quality of this agar prevents saprobic fungi from growing, and the process becomes highly selective for nematode-trapping fungus upon the addition of worms as a separate feeding source. Subsequent research revealed that CMA nutrition modified to 15% was a more successful isolation medium [65]. A soil dilution technique was used in place of the previously described sprinkling plate method to maximize the possibility of isolating nematode-trapping fungus in identical soils [66]. Nematode-trapping fungi are commonly recovered by the soil sprinkling technique [63, 67]. The soil sprinkling method's drawbacks include its labor and time-intensive nature, as well as its inability to determine if the fungi are present in the soil as hyphae or spores [63].

Soil Dilution Method

The Soil Dilution Method is akin to other procedures employed in the isolation of nematode-trapping fungi. This methodology was first presented by [66], who found that the soil dilution technique can generate around ten times more fungal species than the sprinkle plate method when equivalent soils are compared. Currently, nematode-trapping fungi can also be isolated using the soil dilution approach, which was developed from the earlier sprinkling technique [62].

Baerman Funnel Technique

Fungal parasites will typically colonize living nematodes in soils. Thus, nematodes can be extracted from soil and plated

onto low-nutrient agar to isolate nematophagous fungi. The Baermann funnel approach has been employed most frequently, notably for removing nematodes infected by nematophagous fungus (68), despite the fact that other methods have been documented for separating nematodes from the soil [68].

Differential Centrifugation Technique

In microbiology and cytology, it is a standard process to extract individual organelles from whole cells in order to perform additional study on particular cell components [70]. This method has also been modified for the analysis of nematophagous fungus. The tiny endoparasitic fungal conidia are left in the supernatant by spinning down the larger predaceous-type conidia at low speeds using the differential centrifugation technique. Consequently, the heavier spores of nematode-trapping fungi and the lighter spores of endoparasites may be separated using this method. After a faster centrifugation of the supernatant, the spore-containing pellet spreads out on plain agar that has been baited with nematodes, which subsequently become infected [3].

Electron microscopy

This technique creates a magnified image of the specimen by illuminating it with an electron beam. Compared to ordinary, non-confocal light microscopes, which are limited by diffraction to about 200 nm resolution and useful magnifications below 2,000x, this method has a greater resolving power because electrons have wavelengths that are about 100,000 times shorter than visible light (photons). Electrons can achieve better than 50 pm resolution and magnifications of up to about 10,000,000x. Electron microscopy comes in two main forms that are commonly used to provide information about surfaces: Scanning Auger Microscopy (SAM) which forms an image from the Auger electrons emitted by a specific element to provide compositional maps of a surface, and Secondary Electron Microscopy (SEM) which forms a direct image of the topographical nature of the surface from all the emitted secondary electrons. To achieve spatial localization, the probe beam is focused in both of these methods. Electron microscopy has been widely employed to observe species and can yield useful morphological information, as well as some amazing images of nematophagous fungi [71].

Molecular Technique

The identification and classification of fungus has been completely transformed by quickly evolving molecular techniques like the polymerase chain reaction (PCR) [72]. Using these methods to identify nematophagous fungus can be useful for getting an accurate and timely identification [5].

Nematophagous as a biological control agent

Numerous advantages arise for the ecology and economy from nematophagous fungi in the soil [73]. Since they are predators, it is possible to tame them by utilizing the amount of accessible prey. This indicates that its prey continuously regulates its population. It also regulates the number of nematodes. This makes a wide variety of flowers possible to flourish, including those that are primarily vulnerable to nematodes. The fungus benefits from the nematode consumption by gaining mass. This pile can serve as a food source for larger species that are further up the food chain. The food web is expanded and nutrient cycling is encouraged by this type of microbial contact. Because there are so many of these nematodes in the world, soil ecosystems have a steady supply of them, which is constantly replenished by soil movement [74].

Nearly every rectangular foot of soil on the planet contains nematodes. Nematodes that cause plant pathogenicity can also be detrimental to crops. Nematodes are a constant nuisance in fields, robbing crops of their vitamins. However, many of these nematodes can be destroyed by nematophagous fungi before they have a chance to do even more harm (Table 2). In order to combat this threat, nematophagous fungi may be further developed into weapons. The mouthpiece that nematodes use to feed on plants is called a stylet, and it looks like a needle. The nematode injects this mouthpiece into its host, consuming its host's nutrition. In addition to depriving the host of nutrients, this feeding process leaves lesions that could serve as points of entry for invasive microbes looking to colonize the plant and transmit illness [75]. The population of these nematodes is not restricted in any way by the quantity of nematophagous fungus present in the soil. They do, however, represent a positive start in the process of developing an IPM strategy to combat the nematodes [46]. Nematophagous is an alternative to anthelmintic drugs that can be used to treat nematode infections in a variety of animals. This is because nematode resistance to drugs is on the rise, and developing new chemical products is becoming more expensive. Additionally, issues with toxicity, environmental pollution, and residue in animal products affect public health [76].

Nowadays, using chemicals or pesticides is the main strategy for managing parasites; however, the widely used chemicals are rapidly losing their effectiveness because of resistance that develops from their indiscriminate employment. Furthermore, because of their residual build-up, pesticides are dangerous to people, the environment, and non-target animals, birds, helpful insects, and even the crop itself [77]. Natural or biological control is an effective way to deal with the growing problem. Under ideal conditions, biocontrol provides sustainability, something that other methods of controlling parasites do not. Biological control over parasites can be achieved through various means, including the use of parasites (parasitoids), pathogens (such as fungi, bacteria, viruses and virus-like particles, protozoa, and nematodes), and predators (such as fish, birds, rodents, amphibians, flies, beetles, mites, and arthropods) [78].

Compared to other insect and nematode management methods, including chemical pesticides and medications, biological control has advantages. Among these benefits are the following: (1) BCAs are often extremely host specific; (2) there are no residues; (3) it could be economical; (4) it is easy to apply; (5) it is quickly established; (6) it is safe for the applicator and the environment; (7) nematode does not acquire resistant against BCAs, unlike chemical approaches [79]. The drawbacks of biocontrol are as follows: (1) it is frequently slow; (2) BCAs do not eradicate their host; (3) the techniques for shipping, storing, and applying BCAs can be somewhat complicated; (4) the production of the BCA is also expensive in certain situations; (5) biocontrol may occasionally be more expensive than conventional methods; and (6) poorly designed biocontrol may result in drastically altered native biodiversity [80].

The potential of nematophagous fungi as a biocontrol agent

for agriculturally significant nematodes-particularly those that cause gastrointestinal infections in grazing animals-has been studied. Anthelmintic medications are frequently used to treat certain parasite illnesses. Nonetheless, there has been a noticeable rise in anthelmintic resistance. Soil contains the larvae of animal pathogenic nematodes [81]. The use of nematode pathogenic fungus to treat contaminated soils has the potential to lower nematode populations. But after nematode populations are eliminated, the fungus disappears from the soil, which may restrict its application as a long-term biocontrol agent. They lower the infection level on pasture to a point where grazing animals are protected against parasitic nematode-related clinical and subclinical consequences. The number of infectious stages cannot be completely eliminated by any biological control agent; but, since grazing animals, like sheep, regularly come into contact with a limited number of parasite larvae, they ought to be able to mount a defense against them naturally. Under both natural and experimental settings, they have the ability to act as a biological control agent against the free-living stages. These fungi can be found in soil all throughout the world, where they eat a variety of free-living soil nematodes. Duddingtonia flagrans, of the several fungi examined, have the best chance of surviving in the ruminant gut (GIT) by creating sticky, complex traps on their developing hyphae. After leaving the digestive system, spores begin to germinate, and in the fecal environment, growing larval stages are ensnared by looping hyphae. This technology is an environmentally safe biological approach to worm management under sustainable, forage-based feeding systems that has been successfully implemented in field settings with all cattle species [82].

The only way to disperse the fungus spores is to mix them with the extra feedstuffs that are needed on a daily basis. A daily feeding management system is required to ensure that every animal eats the same amount of feed. Larvae in the feces must be sufficiently controlled for at least 60 days throughout the transmission season in order for spores to be nourished. This may be costly and require a lot of time. The goal is to create a bolus prototype that may be administered once and release spores gradually over a period of sixty days. When given to grazing animals on a daily basis, half a million spores stop the animal from losing weight owing to parasite infestation. Spores can only be provided in slightly wet feed block material if the blocks are consumed rapidly due to their extremely short shelf life. Increased output can be achieved by adding 500,000 spores per kilogram of live BW from daily meal [83].

Many anthelmintic medications can be used to treat helminthic disease; however, resistance to anthelmintic medications can develop in animals. When a vulnerable population responds less well to therapy, resistance develops (84). These heritable changes might be genetic (including mutations, deletions, or amplifications of particular genes) or epigenetic (where methylation of genes and promoter areas of the genes modulates the gene expression in response to the drug) [85].

According to general agreement, anthelmintic resistance seems to be a pre-adaptive heritable phenomena, meaning that the gene or genes causing resistance exist in the parasite population even prior to the drug's initial administration. The worm population is exposed to an anthelmintic under these circumstances, which triggers selection and the establishment of resistance. When an animal is exposed to an anthelmintic in the best feasible way, only worms with resistance genes should survive. The resistant survivors are the sole worms laying eggs for a brief period of time (until the animal is re-infected with drug-susceptible worms from pasture), which increases the gene pool for resistance [86]. When a single medication is used frequently and consistently, resistance arises. For example, one drug is taken continuously until it stops working, even though it is usually very effective at first. Although there hasn't been any evidence of a detrimental environmental impact in the few published studies, more research on this crucial topic is still needed. Despite the fact that many experiments have confirmed the potential utility of Duddingtonia flagrans chlamydospores, realistic delivery mechanisms, such as feed-blocks or slow-release devices, must be developed in order to make this tool available for use in future integrated management programs. Biological control, effective grazing management, prudent use of currently available medications, breeds of animals resistant to parasites, bioactive feeds, and maybe vaccinations are a few examples of such control techniques [87].

Conclusion and the way forwarded

Nematophagous fungi are soil-living carnivorous and microscopic fungi that can hunt, penetrate digest and kill nematodes and form biomass. They are living in different agroecological systems, including conventional agriculture, organic farming, and even in natural ecosystems. There are different methods to isolate this fungus such as the baerman technique, soil dilution method, baiting technique, direct plating, serial dilution, spread plate technique, microscopic examination, molecular as well as sequencing technique. They are particularly abundant in soils where there is a history of nematode infestation or where nematode populations are high. They are used as biological control agents for plants, birds, mammals, and human parasitic nematodes. Using nematophagous fungi for controlling pathogenic nematodes from the soil and livestock becomes important integrated parasitic management because they can control without damaging the environment as well as the livestock. Such a biological control method is a sustainable alternative to chemical control since it avoids drug resistance, the problem of toxic residue, and cross-reaction. Thus deworming by anthelmintics should be gradually replaced by biological control. However, further experimental studies should be conducted on the identification, molecular characterization, and efficacy of the nematophagus fungi.

Lists of Abbreviations

AOL: *Arthrobotrys oligospora* lectin
BCA: Biological control agent
BW: Body weight
CMA: Corn Meal Agar
ECM: Extracellular matrix
NTF: Nematode trapping fungus
PDA: Potato Dextrose Agar
SLSP: Subtilisin-like serine proteases
RIPs: Ribosome inactivating protein

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