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## Nematophagous Fungi as an Extraordinary Tool to Control Parasitic Nematodes: A Review

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### Abstract

Due to the harmful impacts of using chemicals in controlling plant pests as nematodes, there is a current trend of employing natural pesticides that show potency together without contaminating the environment or negatively affect human and other creatures. Hence, the name of nematophagous fungi has risen as potent biocontrol tools that attack nematodes specifically without harming surrounding ecosystem. Different nematophagous fungal species act as natural predators of nematodes and soil-dwelling worms. Hence, we aimed in this review to discuss importance of nematophagous fungi, their occurrence, taxonomy and evolution. Also, describing examples of using nematophagous fungi as biological control agents. Moreover, the future of employing nematophagous fungi in general and mushrooms in particular in this field is highlighted.

**Keywords:** Nematophagous fungi; Biological control; Anti-nematodes; Mushrooms; Parasites

### Introduction

The nematodes or roundworms constitute the diverse animal phylum Nematoda. More than 25,000 species have been identified, and more than half of them are parasites (Zhang, 2013). The most important nematodes thereby are the so-called "root-knot nematodes" (e.g., *Meloidogyne* spp.), which are globally distributed and infect more than 2000 plant species and thus reduce global crop yields by about 5% (Park et al., 2014). Men have employed a variety of techniques to get rid of pests that threaten agricultural plants ever since the beginning of agriculture. The most prevalent plant parasitic nematodes are currently managed by the use of resistant cultivars, cultural techniques, and chemical nematicides (Timper, 2014).

In spite of the "successful" use of chemicals to efficiently control plant pests, it has been determined that these compounds are highly hazardous to human health and the environment. So, there is another direct introduction of a biological control agent, i.e., a particular creature that may quickly lower nematode numbers and/or shield the developing seedling from harm (Flint and Dreistadt, 1998). Nematophagous fungi are on the top of the list of natural enemies against nematodes, several species of nematophagous fungi exist in nature that can capture and kill nematodes as natural predators of soil-dwelling worms because they are very effective as biocontrol agents in early and recent studies (Kerry, 2000). They have therefore attracted the attention of scientists as model organisms for "carnivorous and/or eaters" of nematodes and the deciphering of the mechanism used by them to hunt their prey (Van Ooij, 2011). In this review will discuss Nematophagous fungi duo to it is important in agriculture and animal husbandry as biological control.

### Overview of Nematophagous Fungi

A wide range of fungal species known as nematophagous fungus employ their conidia or highly developed mycelial structures to ensnare their prey. More than 200 different taxonomically distinct kinds of nematophagous (nematode-destroying) fungi may all attack living nematodes and use them as food. (Nordbring-Hertz et al., 2006).



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As a result, they are divided into three major types according on how they engage with the animal.

#### ***Endoparasites***

They are mostly obligate parasites and, in most cases, have a restricted host range. Their use for biocontrol is therefore limited (Stirling, 1992). They infect nematodes by their spores, either through ingestion or their attachment to the cuticle (Morton et al., 2004).

#### ***Predators or trap-forming fungi***

The most extensively investigated and utilised species for the biological control of nematodes belong to this category of predatory fungus. The example includes the genera *Arthrobotrys*, *Duddingtonia* and *Monacrosporium*.

#### ***Opportunistic fungi that parasitize eggs, cysts and female nematodes***

Since they were thought to be the most effective agents for reducing nematode and helminth populations because they lower the number of viable eggs in the soil, this group of fungus has been investigated for a very long time (Braga and de Araujo, 2014).

The saprophytic/parasitic abilities of the nematophagous fungus vary. While many fungi that produce traps and eggs may live in soil by themselves, endoparasites are typically more reliant on nematodes for nutrition (obligate parasites). There is a relationship between a certain stage of the fungal mycelium's growth and the capacity to trap nematodes. For instance, nematodes can be mechanically or adhesion-captured using hyphal nets, knobs, branches, or rings. On the other hand, endoparasites use their spores to assault nematodes, which either stick to their surface or ingest them. *Arthrobotrys* spp., such as *A. oligospora*, *A. conoides*, *A. musiformis* and *A. superba*, all form three-dimensional adhesive nets, whereas *A. dactyloides* uses constricting rings to capture nematodes mechanically by the swelling of the ring cells.

#### ***Occurrence of Nematophagous Fungi***

Nematophagous fungi have been found in all regions of the world, from the tropics to Antarctica. They have been reported from agricultural, garden, and forest soils especially abundant in soils rich in organic material. Using the so-called soil sprinkling approach, in which a suspension of nematodes is introduced as bait and 1 g of dirt is sprinkled on the surface of a water agar plate, is an easy way to get nematophagous fungus. The plates are checked for trapped nematodes, trapping organs, and conidia of nematophagous fungus for 5–6 weeks using a low-magnification microscope.

There are 10–15 distinct nematophagous fungal species found in several soils. In most soils, *Arthrobotrys* spp. seem to be widespread. Also, the lower fungi, such as zygomycetes *Stylopaga* spp. and *Cystopaga* spp., and the Chytridiomycete *Catenaria anguillulae* are often found. The largest densities and number of species of nematode-trapping fungi are seen in late summer and fall in temperate agricultural soils, probably as a result of the greater soil temperature and increased input of organic detritus (Persmark et al., 1996).

#### ***Taxonomy and Evolution of Nematophagous Fungi***

All main classes of fungus, including lower (oomycetes, chytridiomycetes, and zygomycetes) and higher fungi, have nematophagous species (ascomycetes, basidiomycetes and deuteromycetes). The majority of nematophagous fungus, including both species that capture nematodes and endoparasites, are deuteromycetes (asexual fungi). The taxonomic position of some of these species has been clarified by the discovery of the corresponding sexual stages of the fungus (Pfister, 1997). For example, the sexual stages (teleomorphs) of a number of *Arthrobotrys*, *Monacrosporium* and *Dactylella* species (anamorphs) have been identified as *Orbilina* spp. belonging to the discomycetes (Ascomycetes). Species of the genus *Nematoctonus* are distinguished from all other nematode-trapping deuteromycetes, not only by being both nematode-trapping and endoparasitic but also by having hyphae with clamp connections, typical for basidiomycetes. As a

result, it has been demonstrated that certain *Nematoctonus* isolates develop fruit bodies that resemble gilled mushrooms (*Hohenbuehelia* spp.).

### ***Nematophagous fungi as biological control agents***

One important aspect of nematophagous fungi is the possibility of using them for biological control of plant- and animal-parasitic nematodes. Plant-parasitic nematodes, are global pests in agriculture, causing severe yield losses. Owing to the ban of many nematicides, e.g., methyl bromide, because of health and environmental concerns, new alternatives for nematode control are therefore needed. There are two general ways of applying biological control of nematodes using nematophagous fungi: addition of large amounts of fungi to the soil or stimulation of the activity of the existing fungi using various amendments.

Because of improved techniques for creating and administering fungal biocontrol agents to soil, as well as greater understanding of the biology of nematode-trapping fungus, there is a resurgence in interest in employing these organisms. Using genetic engineering to boost the pathogenicity and longevity of the imported fungus is one technique to strengthen the control potential of nematophagous fungi. Using genetic transformation, it was possible to generate mutants of the nematode-trapping fungus *A. oligospora* overexpressing a protease gene (PII). Additional copies of the PII gene resulted in the development of more infection structures and faster nematode capture and death rates in mutants (Ahman et al., 2002). By using various genetic markers, it was recently shown that the genetic variation in a worldwide collection of the nematode-trapping fungus *D. flagrans* was very low (Ahren et al., 2004). The information indicates that *D. flagrans* is primarily clonal and that no recombination, not even within the same nation, could be found. Thus, it is unlikely that a mass applied strain of *D. flagrans* will recombine with local isolates.

Also, Bourne et al. (1996), suggest that rhizosphere colonization is necessary for successful establishment, and therefore screening for rhizosphere-competent strains of nematophagous fungi is of paramount importance. Animal-parasitic nematodes cause illness and severe weight loss in livestock all over the world. The chemicals presently used to control these nematodes, anthelmintics, have been shown to develop resistance in the parasitic nematode fauna. A promising approach has been presented in feeding the grazing animals with fungal mycelium containing chlamydospores of nematode-trapping fungi, e.g., *Duddingtonia flagrans*. By allowing the spores to be transported through the animal guts, and grow and produce traps in the faeces and surrounding grass, the fungus then captures newly hatched juveniles of the parasites and reduces the nematode burden in the fields.

In nematophagous fungi, hosts' recognition and adhering to nematode's cuticle or eggshells are the first steps in infection. How fungi penetrate the nematode's cuticle has not been fully elucidated but it was suggested that hydrolytic enzymatic systems (including collagenases, chitinases and proteases) secreted by these fungi play a key role in this process (Yang et al., 2007; Zhang et al., 2020). It was reported that phylogenetic investigation of the pathogenicity-related serine proteases originated from nematophagous fungi have evolved from a common ancestor (Li et al., 2010). The second step in infection process is content digestion, which results in forming fungal biomass both inside and outside the nematodes body. Till now, 5 nematophagous fungi groups were identified based on their infection mechanisms. The first group, is nematode-trapping fungi where host recognition is mediated by lectin, proteins on the fungal surface interacting with sugar molecules on the nematode cuticle (GalNAc, AOL, AofleA, AoMad<sub>1</sub>), a nematode specific pheromone ascaroside, or olfactory mimicry that attracts nematode prey (Hsueh et al., 2013; Zhang et al., 2020). Adhesion exerted by this group starts as a cell-to-cell physical communication between the trap cells and the nematodes (Youssar et al., 2019). It should be noted that volatile compounds such as pyrone, furanone, nitrate, maltol and autophagy are needed to switch from the saprophytism to the pathogenicity during trap formation and adhesion (Chen et al., 2013; Liang et al., 2016; Wang et al., 2018). In this group, the third step (penetration) starts when Fungi pierce the cuticle, form a penetration tube, by the help of both the action of

hydrolytic enzymes and mechanical pressure and extracellular, such as serine proteases, collagenase, and chitinases (Yang et al., 2007 and 2008). The final step (digestion) begins when the nematode content become as lipid droplets, and fungi uptake nutrients from the nematodes content for their reproduction and growth.

In the second group of nematophagous fungi (known as endoparasitic fungi), recognition process is conducted via conidia ingested by the nematodes, or by spores that adhere to the cuticle of the host (Lebrigand et al., 2016). Secondly, adhesion is initiated using adhesive conidia which adhere to the cuticle of the nematode forming an appressorium that presses strongly on the cuticle. Later, motile zoospores encyst on the nematode's surface and germinate to produce an injection tube that help in infecting nematodes by injecting a sporidium (Barron and Dierkes, 1977). Penetration is achieved through enzymatic action, mechanical pressure and strong growth of the trophic hyphae (Jansson et al., 1985; Zhang et al., 2016). Finally, digestion is conducted when new conidiophores are developed from bulbs and press into the internal surface of the cuticle, preventing host nutrients leakage, perturbing the metabolism of the nematode, and eventually causing death of the nematode (Wang et al., 2018). The third group of nematophagous fungi are egg- and cyst-parasitic fungi where aurovertin D shows potent toxicity and recognition of host, while adhesion is initiated by the help of both glycoproteins and appressoria which are responsible for adhesion of conidia and hyphae to the eggshell (Lopez-Llorca et al., 2002). Penetration is achieved using enzymes as proteases and chitinases, while digestion is made after colonizing the nematodal tissues to take nutrients and uses sugars existing in the egg as a carbon source (Larriba et al., 2014). The fourth group of nematophagous fungi are also known as toxin-producing fungi as they induce paralysis via the cilia of nematode sensory neurons during host recognition and adhesion processes (Lee et al., 2020). Then, the fungus penetrates through causing excess calcium influx and hypercontraction of the head and pharyngeal muscle cells in nematodes, while digestion is conducted after toxins cause rapid and systemic necrosis in tissues throughout the nematodal body. The last group of nematophagous fungi are those fungi producing special nematode-attacking devices. In this group, vigorous and sharp projections of the special attack devices, mechanically damage the cuticle of the nematode. The nematode cuticle is then penetrated by a penetration peg that has been specially moulded, and as a result of the enzyme action and mechanical pressure, hyphae populate the worm's interior and protrude from the infected nematode. Finally, digestion process requires toxin assistance to be successful in their nematicidal role (spiny balls) (Zhang et al., 2020).

#### ***Fungi in general and mushrooms especially as Anti-nematode Tool***

Recently many researchers reported that most edible mushrooms have nematocidal activity belong to the genus *Pleurotus* and *Beauveria*, *Ganoderma lucidum*, *Lentinus edodes*, *Cordyceps militaris*, *Metacordyceps neogunnii*, *Hericium erinaceus*, *Dictyophora indusiata*, *Cerioporus squamosus*, *Tirmania nivea*, *Tirmania pinoyi*, and *Agaricus impudicus* (Elkhateeb et al., 2021; Soliman et al., 2022). One of the powerful composts known for creating nematicidal poisonous metabolites is oyster mushroom compost. One of the commercially available oyster mushrooms, the grey oyster (*Pleurotus ostreatus*), is known to contain trans-2-decenedioic acid, a poison released by hyphae that paralyse nematodes upon contact. The anthelmintic activity has been reported from extracts and its fractions obtained from *Pleurotus* fruiting bodies, mycelium, and degraded substrate (Elkhateeb et al., 2021).

#### ***Future of nematophagous fungi***

Huffaker et al. (1971) have proposed certain desirable characteristics of a natural enemy to be effective as a biocontrol agent. Nematophagous fungi does possess some of them. Nematophagous fungi has proven ability to trap and kill nematodes only. As all of them are saprophytic fungi they do survive in absence of nematodes. Most of the species of Nematophagous fungi are widely distributed. Still, isolates from different localities show variability in efficiency of trapping nematodes. These characteristics confirm the potential of Nematophagous fungi as bioagents to control nematodes. A very important drawback of them is that they are affected by antagonism of other soil fungi. This sensitivity to fungi stasis results in

their failure in controlling nematodes in field trials. The future lines of researches that may improve biological management of phytonematodes with Nematophagous fungi include:

- a. Isolation of different species of Nematophagous fungi with three-dimensional sticky network and constructing rings from various localities at national level.
- b. Isolation of predominant soil fungi from same localities.
- c. Study of levels of antagonism or consociation between Nematophagous fungi and other fungi in mixed cultures.
- d. Selection of strains of Nematophagous fungi with better trap-ping efficiency as well as tolerance (compatibility) to other fungi.
- e. Development of fungal bionematicide using selected strain(s) of Nematophagous fungi in carrier based dry, powder form as well as in liquid form for convenience of soil application and longer shelf life (Jackson, 2013). Conidia of all Nematophagous fungi are thin walled, in dry powder form they are not likely to remain viable for longer period.
- f. The fungal bionematicide developed by coordinated work can be simultaneously tried in various localities for different crops.

### Conclusion

To prevent additional harm to the environment and human health, harmful nematicides must be replaced immediately with eco-friendly biocontrol agents. Fungi in general and mushrooms in particular are a potential alternative to these chemicals as they can attack plant-parasitic nematodes without affecting other surrounding creatures. Further studies are encouraged to get full benefits from these fungi and to be capable of targeting harmful pests in a more specific way.

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WAE, DEE and GMD conceived the concept, wrote and approved the manuscript.

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