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Occurrence of Nematophagous fungi in the fresh and spent button mushroom compost in Bihar

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Abstract
The compost samples of button mushroom from Samastipur, Muzaffarpur and Darbhanga districts were processed for the isolation of nematophagous fungi present using serial dilution method on PDA plates. From the button mushroom compost sample of Samastipur district, five fungi *viz*. *Trichothecium roseum*, *Rhizopus stolonifer*, *Geotrichum* spp., *Fusarium* spp. and *Aspergillus niger* were isolated, from Muzaffarpur, only two fungi, *Trichothecium roseum* and *Aspergillus niger* were identified and from Darbhanga samples, two fungi, *Helminthosporium* spp. and *Fusarium* spp. were identified. From spent mushroom compost the fungi identified were, *Trichothecium roseum* and *Geotrichum* spp. From Samastipur district, *Trichothecium* spp. and *Aspergillus niger* from Muzaffarpur district and only *Helminthosporium* spp. was identified from Darbhanga district.
Amongst all the fungi isolated and identified, only three of them were found nematophagous against myceliophagous nematodes. These were *Helminthosporium* spp., *Trichothecium* spp. and *Geotrichum* spp. *Helminthosporium* spp. and *Trichothecium* spp. trapped the nematodes through constricting rings while *Geotrichum* spp. trapped the nematodes through sticky knobs whereas.

Keywords: Button mushroom compost, *Geotrichum* spp., *Helminthosporium* spp., Myceliophagous nematodes, Nematophagous fungi and *Trichothecium* spp.

Introduction
Myceliophagous nematodes are highly destructive and are known to cause damage ranging from 41 to 100% crop loss in mushrooms, depending on the nematode species involved, its population density and the cropping stage at the time of infestation (Sharma *et al.*, 1984; Khanna 1991, 1993 and Khanna & Kumar 2005) [13, 14, 11, 24]. These Nematode infestation reduces crop duration, leads to extremely poor flushes in initial stage followed by total crop failure (Seth 1984; Sharma *et al.*, 1984; Kumar *et al.*, 2008 and Goodey, 1960) [25, 24, 11, 5]. They cause patchy to no growth of mycelium leading to severe reduction in the mushroom yield (Kumar *et al.*, 2008) [11].
The management of these nematodes is difficult due to increase in nematicide cost and environmental problems. Use of nematicides can be detrimental to the human health as mushrooms are consumed fresh. Due to the hazardous effect it has led to an increased interest in biological control in order to achieve environmentally safe method of reducing the nematode damage. As chemical control using effective pesticides have various undesirable plant genic and environmental effects and cause imbalance in microbial dynamics in soil ecosystems (Groth *et al*., 1990) [7], growing ecological concerns has led to intensive research on the alternative methods for the control of plant diseases and biological control of phytopathogenic microorganisms has emerged as one of the most powerful and effective approach (Oka, 2010 and Niu and Zhag, 2011) [20, 19].
Biocontrol is the most relevant and practically damaging approach for the control of nematodes. Amongst the bioagents, fungi constitute a major group of bioagent against various kind of pests. Several fungi were discovered as bioagent against the parasitic nematodes like *Paecilomyces lilacinus* reduced population densities and is considered as one of the most promising and practiced biocontrol agent for the management of plant parasitic nematodes (Jatala, 1985) [8].
Nematophagous fungi are those fungi which trap the nematodes and feed on them causing death of those nematodes. These fungi attack the nematodes by producing some special devices to trap and kill them. These structures may be adhesive hyphae, adhesive nets, adhesive branches, adhesive knobs, non-constricting passive rings and constricting rings. These fungi are very useful in managing plant parasitic and mycophagous nematodes. There were many research works done on different fungi found in soil. Fungi which parasitise nematodes have been studied extensively for the biological control of root feeding, plant parasitic nematodes (Stirling, 1991) [30].

It is already proved that the nematophagous fungi are a good bio-control agent (Kerry *et al.*,1982) [9]. Several work on nematophagous fungi found in soil, have been done. Vyas *et al.* in 1996 [31] observed that *Paecilomyces lilacinus* was effective against *Meloidogyne incognita* in chickpea. Bhardwaj and Trivedi (2000) [2] observed significant reduction in the incidence of *Heterodera cajani* when *P. lilacinus* or *P. chlamydosporia* were applied on cowpea. *Arthrobotrys oligospora*, a nematode trapping fungus has functional nematode capturing devices (Khan *et al.*, 2011 and Simon & Anamika, 2011) [12, 28]. This fungus has potential to reduce disease caused by nematodes through predation.

Trichoderma harzianum was reported to have nematotoxic effects of against *Meloidogyne graminicola* in rice (Pathak and Kumar, 1995) [23]. *T. viridae* inhibited egg hatching and juvenile mortality of *Meloidogyne incognita* (Mayer *et al.*, 2000) [18]. Ansari *et al.* (2002) [1] also reported the adverse effect of *T. viride* against *M. incognita.*

The nematophagous fungi found in mushroom compost, casing soil and spent mushroom compost may be used as a potential bio control agent for the management of mushroom feeding nematodes. Since work on nematophagous fungi found in button mushroom compost is very few, the present study was planned to study the potential predacious fungi from the compost beds, casing soil and spent mushroom compost and its efficacy against the myceliophagous nematodes.

Materials and methods

Isolation of fungi from compost: The compost samples of button mushroom from Samastipur, Muzaffarpur and Darbhanga districts were processed for the isolation of nematophagous fungi present using serial dilution method on PDA plates prepared in sterilized medium. About 1 g of compost from each of three samples was diluted in sterile water @ 10^{-4} dilutions and sprinkled on PDA in Petri plates in 3 replications. These plates were incubated at 25 ^{0}C in BOD Incubator.

Culturing of fungi: After one week of incubation, the Petri plates developed the fungi and bacterial colonies. The individual fungal colonies were cultured separately on PDA Petri plates by using hyphal tip method under sterilized condition. These Petri plates were kept in BOD incubator at 25 ^{0}C. After four days of incubation, the fungi culture was prepared.

Identification of Fungi: The culture of individual fungi was identified using the key of Cooke and Godfrey (1964). This key is based on trapping mechanism and morphology of cavities and spores. Identification was made directly at 400 X magnification. The morphological and cultural characteristics of the fungi were also consulted to make confirmation of the identification.

Culturing Mycophagous Nematodes: The mycophaous nematodes, *Aphelenchus* spp., *Aphelenchoides* spp. and *Ditylenchus myceliophagus* were identified in the button mushroom compost and were cultured on the PDA flasks having *Agaricus bisporus* culture. Twenty individuals of males and females of each mycophagous nematodes extracted from button mushroom compost were inoculated in the *A. bisporus* culture. These flasks were incubated in BOD at 25 ^{0}C. After two weeks, these nematodes fed the fungus completely and multiplied themselves. The mycelia of *A. bisporus* became brown and showing the nematode multiplication. The juveniles and adults were used in the experiment for the screening of predacious fungi. These nematodes were sub-cultured continuously on *A. bisporus* culture for the experiments.

Surface sterilization of nematodes: Before inoculation to the Petri plates, the mycophagous nematodes were sterilized in sterilization tube (Kanwar *et al*., 1994). The tube was immersed in beakers containing 0.1% $HgCl_2$ for 5 minutes followed by 100 ppm of Streptocyclin sulphate and Penicillin for 5 minutes each. After that nematodes were washed three times by dipping the tube in beakers containing sterile distilled water. The sterilized nematodes were collected in beaker by backwashing the tube with a sterile micropippette. These nematodes were used for inoculation in Petri plates having fungal culture.

Nematode inoculation: The surface sterilized nematodes were inoculated in fungal culture plates using a sterile micropippette with a required number of nematodes according to the treatments. The process was done in Laminar air flow chamber under asceptic condition. These inoculated plates were kept in BOD at 25 ^{0}C for 24 hours.

Estimation of predacious fungi from fresh mushroom compost, casing soil and spent mushroom compost: From each of the three districts, the samples of fresh mushroom compost and spent mushroom compost were taken. The isolation of fungi from each of these samples was done separately in PDA Petri plates by serial dilution method at spawning and cropping period stages of button mushroom cultivation. After inoculation, these Petri plates were kept in BOD at 25 ^{0}C. After one week, the fungi were grown and each colony was purified and identified. The identified fungi were cultured separately on PDA Petri plates by hyphal tip method. Again these Petri plates were kept in BOD at 25 ^{0}C for proper growth. These fungi were sub cultured for further experiments. These identified fungi were inoculated with mycophagous nematode, *Aphelenchoides* spp. @ 100 presterilized individuals per plate. After 24 hours of incubation of these plates in BOD at 25 ^{0}C, they were observed under compound microscope by keeping them on glass slides.

Results: The fungi isolated from fresh button mushroom compost, casing material and spent button mushroom compost from Samastipur, Muzaffarpur and Darbhanga districts, were identified and presented in Table 1. The table revealed that there were five fungi species, found in fresh mushroom compost of Samastipur district. These were *Trichothecium roseum*, *Rhizopus stolonifer*, *Geotrichum* spp., *Fusarium* spp. and *Aspergillus niger*. Similarly, only two fungi, *Helminthosporium* spp. and *T. roseum* were identified from Darbhanga district in fresh button mushroom compost.

Table 1: Fungal isolates identified from fresh mushroom compost from Samastipur, Muzaffarpur and Darbhanga districts

S.N.	Samastipur district	Muzaffarpur district	Darbhanga district
1.	*Trichothecium roseum*	*Helminthosporium* spp.	*Trichothecium roseum*
2.	*Rhizopus stolonifer*	*Fusarium* spp.	*Aspergillus niger*
3.	*Fusarium* spp.		
4.	*Geotrichum* spp.		
5.	*Aspergillus niger*		

The fungi from spent button mushroom compost of Samastipur district, were identified as *Trichothecium roseum* and *Geotrichum* sp., from Darbhanga district, the fungi found were *Trichothecium roseum and Aspergillus niger*. From Muzaffarpur district, only one fungus species was isolated from the spent button mushroom compost which was *Helminthosporium* spp. (Table 2).

Table 2: Fungal isolates identified from spent mushroom compost from Samastipur, Muzaffarpur and Darbhanga districts

S.N.	Samastipur district	Muzaffarpur district	Darbhanga district
1.	*Trichothecium roseum*	*Helminthosporium* spp.	*Trichothecium roseum*
2.	*Geotrichum* spp.		*Aspergillus niger*

Morphological and cultural characters of the identified fungi

1. ***Aspergillus* spp.:** The colonies on PDA were initially \white and round to oval in shape. It quickly became black with conidial production and loose in texture. Conidia were brown to black. The hyphae were found septate and hyaline. The conidiophores were varying greatly in length and breadth. The conidial heads split in to columns on maturity.
2. ***Fusarium* spp.:** It grew rapidly on PDA and the colony was white or creamish. The reverse of the Petri plates was coloured or pale yellow in colour. The mycelium was extensive and has cotton like structure. The hyphae were septate and hyaline. The conidiophores were two or more celled, smooth and sickle shaped. The conidiophore was with long chain of macro conidia. The macro conidia was 2-5 celled.
3. ***Trichothecium roseum*:** When grown on PDA as an individual colony, its colony was initially white. It further became light pink to peach colour. Its conidia were light pink. The hyphae were simple. The conidiophores were indistinguishable from vegetative hyphae until production of first conidium. Conidia arise as the blowout from the side of the conidiophore apex.
4. ***Rhizopus* spp.:** The colonies of this fungus grew very rapidly on the plates. The texture was typically cotton candy like. The plate looked pale yellow when seen through the reverse side. The hyphae was broad and non-septate. The sporangiophores were brown in colour and unbranched. Sporangia located at the tip of sporangiophores.
5. ***Geotrichum* spp.:** The colonies grew on plate were seen soft, creamy and white. They were fast growing, matured in 5 days. The hyphae were hyaline and lightly pigmented. Conidiophores were anthrosporous, terminal and inetercalary. The hyphae were dichotomously branched which resembled like tuning fork along the colony margin.
6. ***Helminthosporium* spp.:** The colonies on PDA were seen velvety to woolly on plate. It quickly matured in 5 days. The conidia were olive green to black and it looked black from the reverse side of plate. Hyphae were septate and hyaline conidiophores were brown to dark brown, erect and parallel walled. Conidia were multicellular, large and club shaped. Conidia were located on the side of the conidiophores.

Screening of the identified fungi against nematodes: The isolated and identified fungi, *Helminthosporium* spp., *Trichothecium roseum Geotrichum* spp., *Fusarium* spp., *Rhizopus stolonifer* and *Aspergillus niger* were screened against mycophagous nematodes, *Aphelenchoides* spp. It was found that *Helminthosporium* spp. and *Trichothecium roseum* were able to trap the nematodes by forming a constricting ring. This ring was made up of three cells. The nematodes when enter through this ring, the three cells became bigger in size and nematodes get trapped and the fungus constricted the nematode body at the place of ring. Finally the nematode was consumed by the fungi. The another fungus, *Geotrichum* spp. was also found nematophagous but its trapping mechanism was different (Table 3). Here, it forms sticky knobs and the nematodes when come in contact, these sticky knobs get attached with nematode body and thus the fungus sucked the body fluid through these knobs. The nematodes died after consumption. The trapping mechanism of these nematophagous fungi on different myceliophagous nematodes were shown in Plates 1, 2, 3 and 4.

Table 3: The fungi identified as nematophagous and the type of trap

S.N.	Name of fungi	Type of fungal trap
1.	*Helminthosporium* spp.	Constricting ring (Sticky with three cells)
2.	*Trichothecium roseum*	Constricting ring (Sticky with three cells)
3.	*Geotrichum* spp.	Sticky knobs

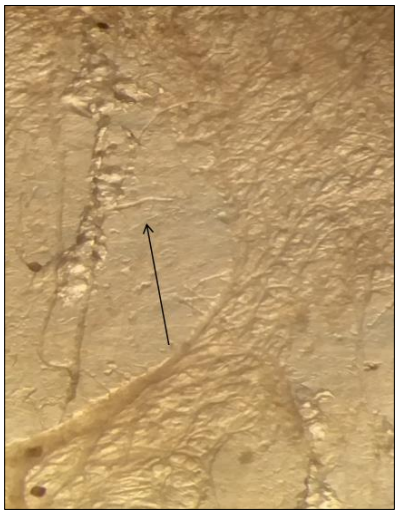

Plate 1: Nematodes trapped by *Trichothecium* spp.

Plate 2: Nematodes trapped by *Helminthosporium* spp.

Plate 3: Nematodes trapped by *Geotrichum* spp.

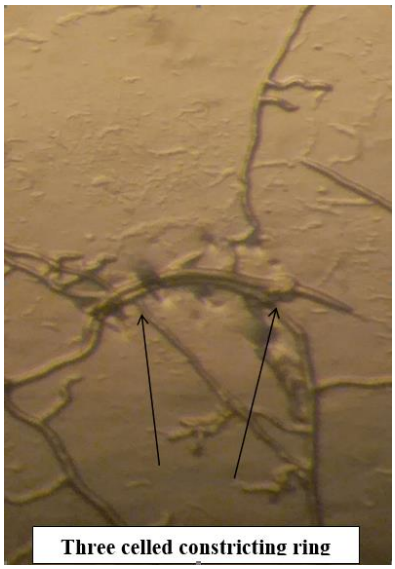

Plate 4: *Aphelenchoides* spp. trapped in *Helminthosporium* spp.

Discussion

There are biotic community present in soil and compost which contain many microscopic organisms which may serve as biological control agent against the plant parasitic or mushroom parasitic nematodes. These organism trap, kill and consume living animals. These may be fungi, bacteria, viruses, and others. The fungi use different devices to trap and kill the nematodes. These are nematophagous fungi.

Nematophagous fungi present in all types of climate and habitats (Gray, 1987 and Dunddington, 1954) [6, 3]. Saxena (2008) [27] reported 16 species of nematode-trapping fungi form Scottland of which *Arthrobotrys gephyropaga* and *Dreschlerella isochopaga* were most common. Singh *et al.* in 2006 reported the presence of nematophagous fungi in the rhizosphere of agricultural plants is important. According to Persmark and Janoson (1997) [21, 22]. Out of 15 nematophagous species, *A. obigospora* is the most common organism in the rhizosphere zone. Application of *A. obigospora* significantly reduced the number of root galls and improved the plant growth in pots which may be due the reduction in number of galls per seedling.

The result is also supported by the findings of Srivastav and Askery (2000) [29] who reported nemataphagous fungi like *Monacrosporium megalosporum*, *Stylopage leiohypha*, *Cystopage cladospora* from the different soil habitats of Pusa Bihar.

Trapping devices used by the nematophagous fungi: Nematode trapping fungi are soil borne microfungi that possess different trapping devices to entrap moving stage of nematodes. These fungi from traps when come in contacts with nematodes. The nematodes are trapped when they nematodes migrate to the traps and the trap attaches to the nematodes surface. In this way the nematodes get killed and digested by the fungi.

Here, the identified nematophagous fungi from the button mushroom compost used constricting ring and adhesive knobs to trap the nematodes. Zopf (1888) [32] was the first to observe the trapping of nematodes by *Arthrobotrys oligospora*. It pierces the nematode cuticle with the help of a penetration tube. The hyphal growth of the fungi takes place at the point where the nematode sticks to the fungi the hypha enters into the nematode body causing an infection bulb at the tip. These spread inside the nematode body and absorb all the contents.

The result is also supported by Liu *et al.,* (2012) [16] that the carnivorous fungi use three celled constricting rings to trap the nematodes. The formation of constricting ring was also reported by Persmark and Nordbring Hertz in 1997 [21, 22]. The most abundant trap devices were net forming species followed by constructing ring and adhesive hyphae (Kim *et al.*, 2001) [10]. The discovery of Li *et al*. in 2006 [15] also supported the formation of non-constricting ring and adhesive knobs by *Dactylellina varietas* and *D. schuanensis*.

Stirling *et al.* (1998) [26] observed that the fungus *A. dactyloides* proliferated and consistently produced traps in soil. *A. Oligospora*, *A. superba* and *D. pseudoclavata* constructed adhesive three-dimensional nets, reported by Mossavi and Zare in 2012 [17]. Dreschsler in 1937 also reported the formation of constricting ring in nematode trapping fungi.

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