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## Efficacy of Nematophagous fungi from button mushroom compost on the myceliophagous nematodes

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**Abstract**

The nematophagous fungi, isolated and identified from button mushroom compost, were screened against three myceliophagous nematodes, *Aphelenchus* spp., *Aphelenchoides* spp. and *Ditylenchus myceliophagus* at five different inoculum levels i.e. at 100, 200, 300, 400 and 500 individuals of nematodes per plate. The number of trapped nematodes and their percentage were calculated. At different inoculum levels of myceliophagous nematodes, *Aphelenchus* spp., *Aphelenchoides* spp. and *Ditylenchus myceliophagus*, fungus, *Helminthosporium* spp. was found to be more efficient to kill the nematodes when compared to *Trichothecium* spp. and *Geotrichum* spp. The number of trapped nematodes increased with increasing inoculum of mycophagous nematodes in all the three nematophagous fungi. But, the maximum percentage of feeding was at 300 and 400 inoculum levels.

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

**Introduction**

Fungi in the form of edible mushrooms represent a wonderful food but some fungi which consume nematodes (Duddington, 1955) [6] are the predacious or nematode-trapping fungi which may be useful for our crop and even for the protection of edible mushroom cultivation. 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) [17].

It is already proved that the nematophagous fungi are a good bio-control agent (Kerry *et al.*, 1982 and Stirling *et al.*, 1979) [11, 18]. Several work on nematophagous fungi found in soil, have been done. Vyas *et al.* in 1996 observed that *Paecilomyces lilacinus* was effective against *Meloidogyne incognita* in chickpea. Bhardwaj and Trivedi (2000) [5] observed significant reduction in the incidence of *Heterodera cajani* when *Paecilomyces 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, 16]. This fungus has potential to reduce disease caused by nematodes through predation. *Trichoderma harzianum* was reported to have nematotoxic effects of *Meloidogyne graminicola* in rice (Pathak and Kumar, 1995) [15]. *T. viridae* inhibited egg hatching and juvenile mortality of *M. incognita* (Mayer *et al.*, 2000) [14]. Ansari *et al.* (2002) [4] also reported the adverse effect of *T. viridae* against *M. incognita*.

The nematophagous fungi were identified from button mushroom compost from Samastipur, Muzaffarpur and Darbhanga districts of Bihar. In the following experiment, the efficacy of these fungi were tested against the myceliophagous nematodes, *Aphelenchus* spp., *Aphelenchoides* spp. and *Ditylenchus myceliophagus* at different inoculums levels.

**Materials and Methods**

**Culturing of fungi:** The fungi isolated from button mushroom compost, were cultured on PDA Petri plates by using serial dilution method and cultured separately on another sterilized PDA plates by hyphal tip method under sterilized condition. These Petri plates were kept in BOD incubator at 25 °C. After four days of incubation, the fungi culture was prepared.

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**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 400X magnification. The morphological and cultural characteristics of the fungi were also consulted to make confirmation of the identification. These fungi were screened against *Aphelenchoides* spp. The fungi which were found nematophagous, were sub-cultured for further experimentations.

**Screening of predacious fungi against mushroom feeding nematodes:** The identified predacious fungi were inoculated with different inoculum levels of each of the mycophagous nematodes, *Aphelenchus* spp., *Aphelenchoides* spp. and *Ditylenchus myceliophagus* as per the following scheduled treatments:

- A (1) *Aphelenchus* spp. @ 100 individuals per plate + identified predacious fungi  
 (2) *Aphelenchus* spp. @ 200 individuals per plate + identified predacious fungi  
 (3) *Aphelenchus* spp. @ 300 individuals per plate + identified predacious fungi  
 (4) *Aphelenchus* spp. @ 400 individuals per plate + identified predacious fungi  
 (5) *Aphelenchus* spp. @ 500 individuals per plate + identified predacious fungi
- B (1) *Aphelenchoides* spp. @ 100 individuals per plate + identified predacious fungi  
 (2) *Aphelenchoides* spp. @ 200 individuals per plate + identified predacious fungi  
 (3) *Aphelenchoides* spp. @ 300 individuals per plate + identified predacious fungi  
 (4) *Aphelenchoides* spp. @ 400 individuals per plate + identified predacious fungi  
 (5) *Aphelenchoides* spp. @ 500 individuals per plate + identified predacious fungi
- C (1) *Ditylenchus myceliophagus* @ 100 individuals per plate + identified predacious fungi  
 (2) *Ditylenchus myceliophagus* @ 200 individuals per plate + identified predacious fungi  
 (3) *Ditylenchus myceliophagus* @ 300 individuals per plate + identified predacious fungi  
 (4) *Ditylenchus myceliophagus* @ 400 individuals per plate + identified predacious fungi  
 (5) *Ditylenchus myceliophagus* @ 500 individuals per plate + identified predacious fungi

All treatments were kept in BOD at 25 °C for 24 hours. After that, the plates were filled with sterile water @ 10 ml per plate and kept for half an hour at room temperature. From these plates, the nematodes with water, were poured in counting dish and observed under stereoscopic microscope. During observation, the number of live nematodes were counted which were not trapped by the fungi. For accuracy, the individual Petri plates were also observed under the microscope and carefully counted the live nematodes left in the plate. The total number of live nematodes per plate was subtracted from the number of nematodes initially inoculated. This gave the total number of trapped nematodes. By this method, the whole observation was done and the data were analysed statistically. The percentage of nematodes trapped was also calculated and presented in Tables.

## Results

### Screening of the potential predacious fungi against mushroom feeding nematodes

**Against *Aphelenchus* spp.:** The screening of potential predacious fungi, *Helminthosporium* spp., *Trichothecium roseum* and *Geotrichum* spp. was done against *Aphelenchus* spp. at different inoculum levels of nematode to see the efficiency of nematophagous fungi. The data were recorded after 24 hours of inoculation and it was presented in Table 1. The table shows that all the treatments were significantly different to each other. As the inoculum level increases from 100 to 500 per plate, the number of trapped nematodes increased in all the three nematophagous fungi, *Helminthosporium* spp., *Trichothecium roseum*. and *Geotrichum* spp. But the number of trapped nematodes was more in case of *Helminthosporium* spp. followed by *Trichothecium* spp. and lowest trapped nematodes were found in *Geotrichum* spp. Although the number of trapped nematodes increased with inoculum level but the percentage of trapped nematodes in case of *Helminthosporium* spp. decreased from 82.66% at the inoculum level of 100 individuals of *Aphelenchus* spp. to 78.16% at 200 individuals of *Aphelenchus* spp. but again increased to 87.67% at 300 inoculum level, decreased to 78.72% at 400 and increased to 83.40% at 500 inoculum level. The maximum percentage of feeding (87.67%) was seen at the inoculum level of 300 individuals of *Aphelenchus* spp. per plate of *Helminthosporium* spp. culture (Figure 1).

In case of fungus, *Trichothecium roseum*, the number of trapped nematodes increased from 8.26 to 20.52 when the inoculum level of *Aphelenchus* spp. increased from 100 to 500 individuals per plate. The maximum trapping of nematodes was done by the fungus, *Trichothecium roseum* was at 500 inoculum level. But, when the percentage of trapped nematodes was calculated, the maximum percentage of feeding was at the inoculum level of 400 individuals of *Aphelenchus* spp. per plate. The percentage of trapped nematodes increased from 67.33 to 72.66 when the inoculum level increased from 100 to 300 individuals per plate. At highest inoculum level i.e., at 500 individuals of nematodes, the percentage of trapped nematodes was 84.00 which was lower compared to the inoculum level of 400 nematodes where the nematodes trapped was 89%. The optimum level of inoculum was 400 nematodes where the maximum percentage of nematodes were trapped.

When the nematodes were inoculated from 100 to 500 per plate in the culture plates of *Geotrichum* spp., the number of trapped nematodes increased from 7.57 to 19.89 being the maximum consumption at 500 level of inoculum. But, the percentage of trapped nematodes increased from 56.33 at the inoculum level of 100 individuals of *Aphelenchus* spp. to 82.66% at 400 inoculum level of nematodes and the maximum consumption of nematodes by the fungus, *Geotrichum* spp. was at 400 inoculum level. At 500 individuals of *Aphelenchus* spp., the trapped nematodes by *Geotrichum* spp. was decreased to 78.93% from 82.66% which was at lower level of 400 inoculum level as shown in Figure 1.

**Against *Aphelenchoides* spp.:** The mixed populations of *Aphelenchoides* spp. at the inoculum levels of 100, 200, 300, 400 and 500 individuals per plate was inoculated with the potential nematophagous fungi, *Helminthosporium* spp.,

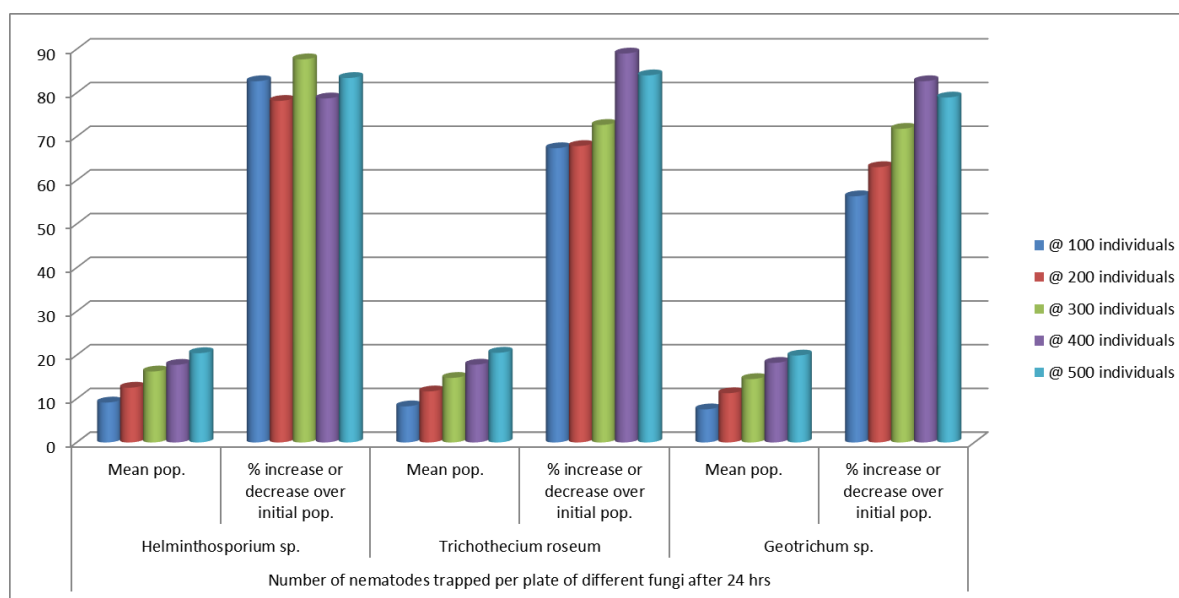
*Trichothecium roseum* and *Geotrichum* spp. and the number of trapped nematodes was estimated after 24 hours of inoculum and the data were presented in Table 2. The percentage of trapped nematodes was also calculated and shown in the table. From the table, it was found that the number and percentage of trapped nematodes were more in the fungus, *Helminthosporium* spp., followed by *Trichothecium roseum*. and was lowest in case of *Geotrichum* spp. culture plates. The number of trapped nematodes increased with the increase of inoculum level of nematodes from 100 to 500 individuals per plate of fungus culture of all the three fungi.

In case of *Helminthosporium* spp., the number of trapped nematodes was 8.34 at 100 inoculum level which increased to 11.57, 14.70, 17.26 and 18.93 at 200, 300, 400 and 500 inoculum levels of *Aphelenchoides* spp. respectively. The maximum highest trapped nematodes were found at the highest inoculum level of 500 individuals per plate. But, this trend was not seen when the trapped nematodes were calculated in percentage. The trapped percentage of nematodes was decreased from 68.66 to 66.50 when the inoculum level increased from 100 to 500 nematodes per plate. Then it increased to 71.77% and 74.5% at 300 and 400 levels of inoculum of nematodes and then it decreased to 71.73 at 500 inoculum level (Figure 2).

**Table 1:** Screening of nematophagous fungi against *Aphelenchus* spp. (Average of three replications)

Treatments <i>Aphelenchus</i> spp. per plate	Number of nematodes trapped per plate of different fungi after 24 hrs					
	<i>Helminthosporium</i> sp.		<i>Trichothecium roseum</i>		<i>Geotrichum</i> sp.	
	Mean Population	Percentage increase (+) or decrease (-) over initial population	Mean Population	Percentage increase (+) or decrease (-) over initial population	Mean Population	Percentage increase (+) or decrease (-) over initial population
@ 100 individuals	82.66 (9.14)	82.66	67.33 (8.26)	67.33	(7.57) 56.33	56.33
@ 200 individuals	156.33 (12.53)	78.16	135.66 (11.68)	67.83	(11.27) 126.00	63.00
@ 300 individuals	263.00 (16.24)	87.67	217.33 (14.77)	72.66	(14.50) 215.33	71.77
@ 400 individuals	314.66 (17.76)	78.72	316.00 (17.80)	89.00	(18.21) 330.66	82.66
@ 500 individuals	417.00 (20.43)	83.40	420.33 (20.52)	84.00	(19.89) 394.66	78.93
CD (0.05)	0.89		0.59		0.53	
SE (m)	3.19		0.18		0.16	
CV	0.28		2.22		2.01	

Data in parentheses are square root transformed values of nematode population

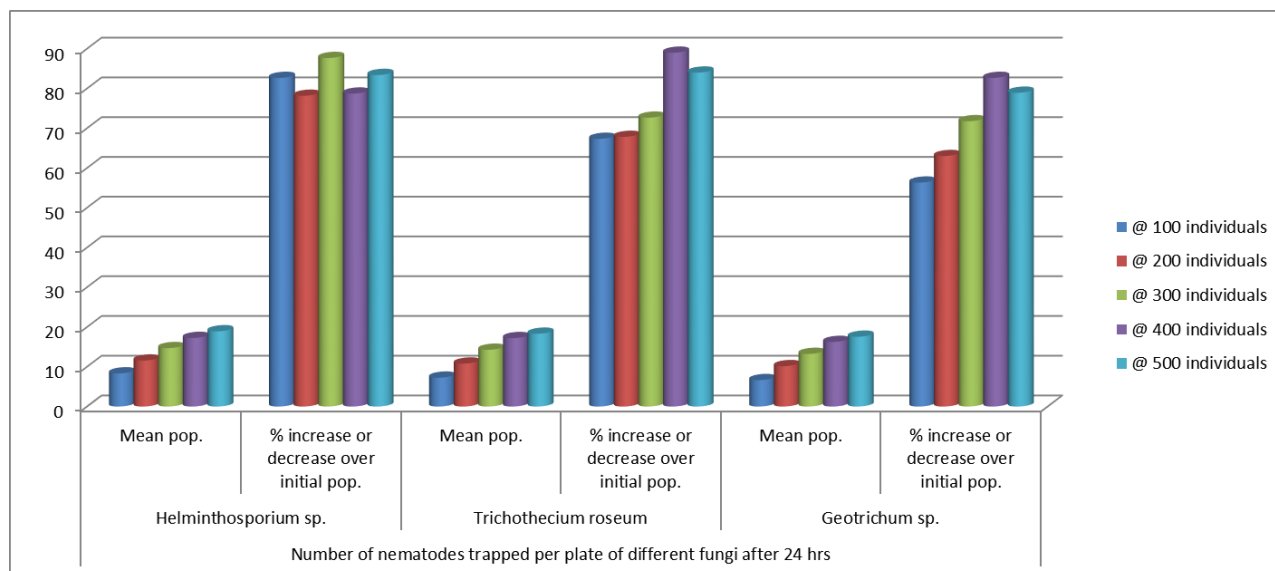


**Fig 1:** Number of nematodes, *Aphelenchus* spp. trapped by the three fungi and the percentage increase and decrease of trapped nematodes over initial population

**Table 2:** Screening of nematophagous fungi against *Aphelenchoides* spp. (Average of three replications)

Treatments <i>Aphelenchoides</i> spp. per plate	Number of nematodes trapped per plate of different fungi after 24 hrs					
	<i>Helminthosporium</i> sp.		<i>Trichothecium roseum</i>		<i>Geotrichum</i> sp.	
	Mean population	Percentage increase (+) or decrease (-) over initial population	Mean population	Percentage increase (+) or decrease (-) over initial population	Mean population	Percentage increase (+) or decrease (-) over initial population
@ 100 individuals	68.66 (8.34)	68.66	52.00 (7.27)	52.00	43.33 (6.65)	43.33
@ 200 individuals	133.00 (11.57)	66.50	116.00 (10.81)	58.00	102.33 (10.16)	51.16
@ 300 individuals	215.33 (14.70)	71.77	203.00 (14.28)	67.67	174.33 (13.24)	58.11
@ 400 individuals	297.00 (17.26)	74.50	295.66 (17.22)	73.91	263.33 (16.25)	65.83
@ 500 individuals	357.66 (18.93)	71.53	335.00 (18.32)	67.00	307.33 (17.56)	61.46
CD (0.05)		0.46		0.58		0.47
SE (m)		0.14		0.26		0.15
CV		1.76		2.32		1.98

Data in parentheses are square root transformed values of nematode population



**Fig 2:** Number of nematodes, *Aphelenchoides* sp. trapped by the three fungi and the percentage increase and decrease over initial population

**Against *Ditylenchus myceliophagus*:** The potential predacious fungi, *Helminthosporium* spp., *Trichothecium roseum* and *Geotrichum* spp. were screened against *Ditylenchus myceliophagus* at different inoculum levels of 100, 200, 300, 400 and 500 individuals per plate. The data of trapped nematodes were recorded in each case.

The number of trapped nematodes was more in *Helminthosporium* spp. followed by *Trichothecium roseum* and *Geotrichum* spp. The number of trapped nematodes increased with the increase in inoculum level of *Ditylenchus myceliophagus* in each culture of fungi, *Helminthosporium* spp., *Trichothecium roseum* and *Geotrichum* spp. All the treatments recorded the highly significantly different result with each other, when the individuals of *D. myceliophagus* were inoculated @ 100/plate with *Helminthosporium* sp., the trapped nematodes were 96 which increased to 184.33 at 200 inoculum level of nematodes. Although the number was increased but the percentage of trapped nematodes decreased from 96% to 92.16% at 300 inoculum level of nematodes. The trapped nematodes increased slightly from 92.16% to 92.74%. This increase was again recorded when the nematodes were inoculated at the rate of 400 individuals/plate. It was 95.75% which was the maximum percentage of trapped nematodes, again at inoculum level of 500 individuals of nematodes/plate showed a slight decrease from 95.75% to 94.46% but the number increased from 383 at 400 inoculum level to 472.33 at 500 inoculum level. The maximum trapping of nematodes

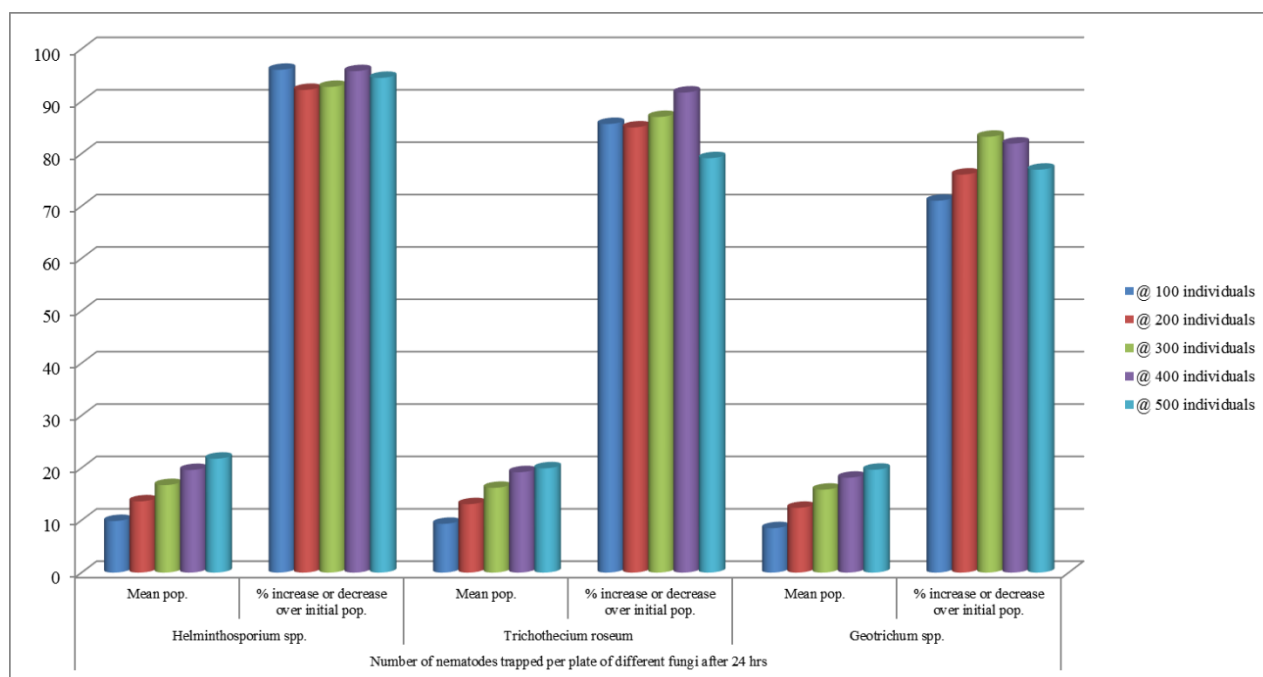
was done at 400 inoculum level of nematodes by *Helminthosporium* spp. In case of fungus, *Trichothecium roseum*, the number of trapped nematodes was 85.66 at 100 individuals of *D. myceliophagus* which increased to 170.00 at the inoculum level of 200/plate. The data of per cent trapped nematodes decreased slightly from 85.66 to 85.00 at 300 and 400 inoculum levels. The number of trapped nematodes increased to 261.00 and 366.66 respectively and the percentage also increased from 85.00 to 87.00 and 91.66 respectively. This percentage decreased from 91.66 to 79.13 at highest inoculum level of 500/plate, although the number increased from 366.66 to 395.66. The trapped nematodes at each inoculum level were highly significantly different except in 400 and 500 inoculum levels where the number of nematodes trapped was different significantly but not very highly different. In *Trichothecium roseum*, the maximum trapping of nematodes was recorded at 400 inoculum level.

In case of *Geotrichum* spp., the number of nematodes trapped was 71.00 at 100 inoculum level. This number increased from 71.00 to 152.00, 249.66, 327.66 and 384.66 at 200, 300, 400 and 500 inoculum levels respectively. When the percentage of trapped nematodes was calculated, it increased from 71.00 to 76.00 and 83.22 at 200 and 300 inoculum levels respectively but it then decreased from 83.22 to 81.91 and 76.93 at 400 and 500 inoculum levels respectively. In this case, maximum trapping of nematodes was done at 300 inoculum level of *D. myceliophagus* individuals /plate.

**Table 3:** Screening of nematophagous fungi against *Ditylenchus myceliophagus* (Average of three replications)

Treatments <i>Ditylenchus</i> spp. per plate	Number of nematodes trapped per plate of different fungi after 24 hrs					
	<i>Helminthosporium</i> spp.		<i>Trichothecium roseum</i>		<i>Geotrichum</i> spp.	
	Mean population	Percentage increase (+) or decrease (-) over initial population	Mean population	Percentage increase (+) or decrease (-) over initial population	Mean population	Percentage increase (+) or decrease (-) over initial population
@ 100 individuals	96.00 (9.85)	96.00	85.66 (9.31)	85.66	71.00 (8.48)	71.00
@ 200 individuals	184.33 (13.61)	92.16	170.00 (13.07)	85.00	152.00 (12.37)	76.00
@ 300 individuals	278.33 (16.71)	92.74	261.00 (16.18)	87.00	249.66 (15.83)	83.22
@ 400 individuals	383.00 (19.59)	95.75	366.66 (19.17)	91.66	327.66 (18.12)	81.91
@ 500 individuals	472.33 (21.75)	94.46	395.66 (19.91)	79.13	384.66 (19.64)	76.93
CD (0.05)		0.33		0.42		0.55
SE (m)		0.10		0.13		0.17
CV		1.09		1.48		2.02

Data in parentheses are square root transformed values of nematode population



**Fig 3:** Number of nematodes, *Ditylenchus myceliophagus* trapped by the three fungi and the percentage increase and decrease over initial population

## Discussion

Biological control is an important tool for pest and disease management. There are numerous organisms exist with antagonistic activity against plant parasitic nematodes (Sterling 1991) [17]. Various aspect of biological control of nematodes using fungi have been reviewed by Jafee (1992) [8], Siddiqui and Mahmood (1996) [19] and Kerry (2000) [9], *P. chlamyosporia*, a facultative egg parasite of cyst and root knot nematodes is a potential bicontrol agent against nematodes (Kerry 2000, 2001) [9, 10].

The comparative efficacy of nematophagous fungi was supported by finding of Aboul-Eid in 1963 [2] and Aboul-Eid *et al.*, in 1997 [3] and 2002 [1] who demonstrated that the efficacy of various fungi differs in trapping and parasitizing nematodes. They found that *A. Dactyloides* was more efficient when *Dactylella brochopaga* and *Monacrosporium eudermatum* in trapping the nematodes, *Meloidogyne graminicola*. Another finding was also in support of this results. Zouhar *et al.*, in 2010 [21] evaluated six strains of nematopathogenic fungi. *A. obigospora*, *Dactylella oviprasitica*, *Dactylellina candida*, *Dactylellins lysipaga*, *Dactylellina phymatopage* and *P. chlamyosporia* against these species of plant parasitic nematodes, i.e., *Ditylenchus dipsaci*, *Globodera rostochiensis* and *Meloidogyne hapla*, *A. oligospora* proved the most pathogenic fungus to all three tested species of nematodes.

The nematophagous fungi, *Hirsutella rhossiliensis* and *Verticillium balanoides* reduced the population of *Ditylenchus dipsaci* in white clover (Hay and Betson 1997) [7]. Khan *et al.* (2012) [13] again supported the nematophagous fungi in this results that nematophagous fungi, *Pochonia chlamyosporia*, *Paecilomyces lilacinus*, *Trichoderma harzianum* reduced the suppressive effect of nematodes, root-knot on egg plant.

Although the pesticide provides a proper and early control of mushroom diseases caused by nematodes, the risk of pesticide resistance, environmental impact and residual persistence would be potential problem. The combination of biocontrol agent and chemical pesticide could both reduce the risk of the occurrence of pesticides resistance and improve the reliability of diseases control. But, in case of mushroom cultivation, one

should rely only on the use of biocontrol agents and that only which are present in compost like nematophagous fungi, predatory nematodes etc. which can easily be cultured and multiplied because they are facultative parasites. They can feed on nematodes as well as on saprophytic microorganisms. The further research may add more information and may help the mushroom growers to keep produce their yield chemical residue free and also save the environment.

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