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In-vitro screening of nematode trapping fungi against root knot nematode

Sumit Kumar Pandey, Prahlad Masurkar and RK Singh

Abstract
The nematode trapping fungi comprise more than 700 species found in all major taxonomic groups. The nematode trapping fungi, which infect nematodes with their hyphal traps or conidia, are more dependent on nematodes as a nutrient and considered as obligate parasites. In this study nine species of nematode trapping fungi were compared with different experiment *in vitro* viz. effect of temperature, effect of different media, effect of different pH, effect of carbon nitrogen sources for their growth. In all the experiment conducted *in vitro* and was found that the most potent nematode trapping fungi as *Arthrobotrys oligospora* followed by *Monacrosporium eudurmatum* and least growth was observed in *Dactylaria brochopaga*. Among these species potential of predacity and trapping structure induced by nematode trapping fungi in the presence of root knot nematode was also assessed and found *Arthrobotrys oligospora* killing 49% root knot nematodes (*Meloidogyne incognita*) and trapping structures induced by this fungi was 2+ (26-50 traps) after 96 hours. The least predacity was observed in *Dactylaria brochopaga* having predacity of 12% with similar number of induced trapping structures i.e. 2+ (26-50 traps) observed after 96 hours.

Keywords: NTF, *Arthrobotrys oligospora*, *M. eudurmatum*, *Dactylaria brochopaga*, *Meloidogyne incognita*

Introduction
The nematode trapping fungi comprise more than 700 species found in all major taxonomic groups including lower (oomycetes, chytridiomycetes, zygomycetes) and higher fungi (ascomycetes, basidiomycetes and deuteromycetes) (Barron, 1977; Waller and Larsen, 1993) [1, 21]. This group of fungi produces extensive hyphae in the natural environment and is able to live either saprophytic or parasitic life (Gray, 1987) [6]. By infecting nematodes the predacious fungi have the ability to thrive in a sheltered environment protected from other soil microorganisms. The nematode trapping or predaceous fungi, endoparasitic fungi, and fungi that produce metabolites toxic droplets to kill nematodes (Stirling 1991) [19]. Morphological feature of most of the nematode trapping fungi that capture and kill nematodes was described by Drechsler, Barron and Liu. Nematode trapping fungi (NTF) can grow as saprophytes in soils, and enter the parasitic stage by developing specific traps. These sophisticated hyphal structures, such as hyphal nets, knobs, branches or rings, in which nematodes are captured by adhesion or mechanically entaglement. (Nordbring-Hertz *et al*., 2004; Singh, 2007) [13, 17]. Therefore, the formation of traps by NTF is an important indicator of their switch from a saprophytic to a predacious lifestyle (Yang *et al*. 2011) [22]. Due to extremely low nitrogen levels, nematode trapping fungi have evolved parasitic behavior to satisfy their nitrogen requirements as nutritional demands (Thorn & Barron, 1984; Barron, 2003) [2].
The nematode trapping fungi, which infect nematodes with their conidia, are more dependent on nematodes as a nutrient and are considered as obligate parasites (Jansson & Nordbring-Hertz, 1979; Jansson, 1982) [7, 8]. Most nematode-trapping fungi can live both saprophytic ally on organic matter and as predators by capturing tiny animals. Nematode trapping fungi are important natural enemies of nematodes in soil. In recent years, environmental and health concerns over the use of inorganic nematicides have greatly increased the demand for the development of biological control agents in plant protection. The reason for the growing interest in nematode trapping fungi is mostly their potential as biocontrol agents against plant- and animal-parasitic nematodes. Nematode-trapping fungi are usually not host specific and can trap all soil-dwelling nematodes. The present study was made to screening the potent nematode trapping fungi against root knot of nematode in *in-vitro*.

Material and Methods

Effect of temperature and culture media on growth and multiplication of nematode trapping fungi

The radial growth of different isolates of Nematode trapping fungi was studied on corn meal agar medium at different temperatures. 5 mm fungal disc of each isolate of nematode trapping fungi was taken from the periphery of 7 day old cultures and inoculated separately into Petri dishes. The inoculated Petri dishes were incubated at temperatures viz. 20, 25, 30 & 35 ^{0}C. Radial growth of the fungus was measured daily until some petri dishes were fully covered by fungal growth or up to 5 days.

The radial growth of all nematode trapping fungi was studied on ten different medium: Nurtrient agar medium, Martin's agar medium, Beef extract agar medium, Sabouraud's agar medium, Malt extract agar medium, Richard's agar medium, Czapek dox agar medium, Potato Dextrose agar medium, Thornton agar medium, Corn meal agar medium.

Effect of pH on growth and multiplication of nematode trapping fungi

Radial growth of Nematode trapping fungi was studied on corn meal agar medium and potato dextrose agar medium at different pH. The medium was prepared and their different pH were maintained with the help of HCl and NaOH solution than mycelial disc of nematode trapping fungi was taken and inoculated separately into petri dishes at different pH viz, 4, 5, 6, 7, 8, 9 and 10. Three replications were maintained for each treatment. Radial growth of the fungus was measured daily until some petri dishes were fully covered by fungal growth or up to five days. The experiment was conducted in complete Randomized Design and data were analyzed statistically.

Nutritional requirement of nematode trapping fungi

The utilization of carbon and nitrogen sources by nematode trapping fungi was studied on basal media i.e. a modified medium found to support growth of these fungi according to Blackburn and Hayes (1966). The carbon sources used were Sucrose, Dextrose, Maltose and Glucose which four nitrogen sources were Sodium nitrate, Potassium nitrate, Ammonium chloride, Ammonium nitrate. The composition of basal medium used for this experiments contain KH_2PO4 - 0.30 gm, $MgSO_4.7H_2O$ – 0.060 gm, KCL – 0.060 gm, $FeCl_3$ – 0.003 gm, $ZnSO_4.4H_2O$ – 0.003 gm and distilled water 1 liter.

Carbon Utilization

Four carbon sources were added to the basal medium. The amount of carbon equivalent to 10 g maltose per liter was calculated to be 4.2 g/L. The different carbon sources were added to provide 4.2 g of carbon per liter. In each flask containing basal medium, carbon sources were added separately. The growth was estimated as a dry mass of mycelial mats of cultures grown for 25 days at 25^0 C.

Nitrogen utilization

For the utilization of nitrogen sources four nitrogen compounds were supplemented to the basal medium as a sole source of nitrogen. The mass of different nitrogen sources was calculated so as to provide 0.3294 g nitrogen per liter which was equivalent to that present in the basal medium. In erlenmeyer flasks of 150 ml in 25 ml of basal medium along with single, nitrogen sources was added separately. The cultures were grown for 25 day at 25^0 C and the growth was estimated as a dry mass of mycelial mats. A control set was prepared with no nitrogen sources in basal medium.

In-vitro evaluation of nematode trapping fungi on the basis of predacity and trapping structure

The screening of potent nematode trapping fungi was done on the basis of trapping structure and predacity. The predacity of all the isolates of nematode trapping fungi were tested against 2nd stage juveniles of *Meloidogyne incognita.* 2nd stage juvenile of *M. incognita* was collected from the egg mass of tomato roots. Egg masses were picked up from the roots using dissecting needle and forceps. Predacity of nematode trapping fungi against plant parasitic nematode was tested by the method describe by den Belder and Jansen (1994) [3]. Desired population of second stage juvenyle were collected in cavity blocks and thoroughly rinsed five times with sterile distilled water. For interaction studies of all the nematode trapping fungi and nematodes in dual culture. A small drop of water containing 100 nematodes was applied into each Petri dish with the help of sterilized dropper. Nematode inoculated Petri dishes were incubated at 25±1^{0}C. Observations on number of trapped nematodes were recorded up to 96 hrs The percentage of captured nematodes was calculated and number of trapping structure or devices induced in response to plant parasitic nematodes was recorded up to 96 hrs.

Result

Effect of temperature and culture media on growth and multiplication of nematode trapping fungi

Observation has been taken for the radial growth and sporulation of nematode tapping fungi at 20^{0}C (fig.-1). The maximum radial growth was observed during the 5th day of inoculation in all nematode tapping fungi species. Among the *Arthrobotrys* spp. *A. oligospora* shows maximum radial growth i.e. 63.2 mm followed by *A. muciformis* i.e. 56.3mm, while the minimum radial growth was observed in *A. conoides*. In the *Monacosporium* spp, *M. eudermatum* showed maximum radial growth of 59.9 mm followed by *M.* 57.5mm. While the minimum radial growth was observed in *M. haptotylum* 42.1 mm. The nematode tapping fungi *D. brochophaga* showed 37.4 mm growth which was maximum at 5th day of inoculation

The radial growth observed at 25˚C (fig-2). Among the *Arthrobotrys* species, *A. oligospora* shows 86.5 mm radial growth during 5th day of observation followed by 77.3 mm by *A. muciformis*. Another nematode tapping fungi spp *Monacosporium* also taken under observation at 25˚. *M. eudermatum* showed 81.2 mm radial growth which was maximum at 5th day followed by *M. cyanopahaga* 72.5 radial growth. Minimum radial growth was observed in *M. gephyropagum* with 55.3 mm growth. The nematode tapping fungi *D. brochophaga* showed 49.4 mm growth which was maximum at 5th day of inoculation.

At the temperature 30˚C (fig.- 3) the maximum growth and sporulation was observed in the *A. oligospora* as 76.3 mm at 5th day of inoculation followed by the *A. superba* as 51.5mm, whereas in the another species of *Arthobotrys*, *A. conoides* showed least radial growth i.e. 42.2mm. In the *M. eudermatum* showed maximum radial growth i.e. 64.1 mm followed by *M. gephyropagum* as 59.1 mm at 5th day of inoculation. The nematode tapping fungi *D. brochophaga* showed 36.4 mm growth which was maximum at 5th day of inoculation.

The parameter of growth and sporulation of nematode tapping fungi (fig- 4) was taken at 35˚C. Among the *Arthrobotrys* species, *A. oligospora* shows maximum radial growth of 43.2 mm followed by *A. muciformis* 26.8 mm at 5th days of inoculation. The minimum radial growth was observed in *A.*

conoides i.e. 20.4 mm. Another nematode tapping fungi *Monacrosporium* species, *M. eudurmatum* shows maximum radial growth i.e. 39.9 mm followed by the *M. gephyropagum* as 30.4 mm. the least radial growth was observed in *M. haptotylum* at 5th day of inoculation. Another species *D. brocophaga* shows least radial growth of 15.5 mm from all the nematode tapping fungi observed at 35˚C.

There was found significant difference in the radial growth on 5th day observation between *A. oligospora*, *M. eudermatum* and *D. brochopaga* on corn meal agar, potato dextrose agar, czapek dox agar, richard'sa agar, sabouraud's agar and malt extract agar media.

Ten different media was tested for screening the suitable media for the growth and sporulation of all the selected nematode trapping fungi (fig.-5). On the basis of daily observation of radial growth corn meal agar media was observed better growth which is suitable for growth and sporulation of nematode trapping fungi.

Among *Arthrobotrys* species maximum growth was reached of *A. oligospora* as 82.7 mm on corn meal agar media on 5th day of observation followed by potato dextrose agar media as 70.6 mm. The minimum growth of *A. oligospora* was recorded for the medium czapek dox agar as 25.2 mm followed by 27.5 mm as richard's agar and as 29.6 mm on sabouraud's agar.

The growth and sporulation of all the selected nematode trapping fungi on corn meal agar media was better than *A. suprba* and *A. conoides.* The better growth and sporulation of *A. suprba* and *A. conoides* was recorded on potato dextrose agar media as 68.36 mm and 60.57 mm respectively.

Among the growth of *Monacrosporium* species better growth of *M. eudermatum* was observed on corn meal agar media as 74.8 mm followed by potato dextrose agar as 63.3 mm. The minimum radial growth of *M. eudermatum* was observed on czapek dox agar as 22.9 mm, richard's agar as 24.8 mm, malt extract agar as 26.3 mm and 27.1 mm on sabouraud's agar as.

Among all the nematode trapping fungi lowest growth was recorded of *D. brochopaga* on corn meal agar media as 40.6 mm while growth on potato dextrose agar as 32.5 mm followed by sabouraud's agar as 10.5 mm, czapek dox agar as 10.2 mm, richard's agar as 12.6 mm and malt extract agar as 12.1 mm.

The radial growth of nematode trapping fungi was studied on corn meal agar medium at different temperatures: 20, 25, 30 & 35 ^{0}C. Corn meal agar media and potato dextrose agar media which were found very good for growth and sporulation of nematode trapping fungi which the optimum temperature was found good at 25-30 ^{0}C for better growth decrease growth was recorded decreasing with higher temperature. Nematode-trapping fungi can be easily grown on various artificial media (Belder and Jansen 1994; Faust and Pramer, 1964) [3, 5].

Similar result was also found by Sanyal, (2000) [14] and Mukhopadhyaya *et al.* (2001) [10] who observed that the growth and sporulation of nematodes trapping fungi on and temperature and concluded that corn meal agar, a semi-synthetic medium, was best suited for growth and also observed the optimum growth of nematode trapping fungi at 26°C.

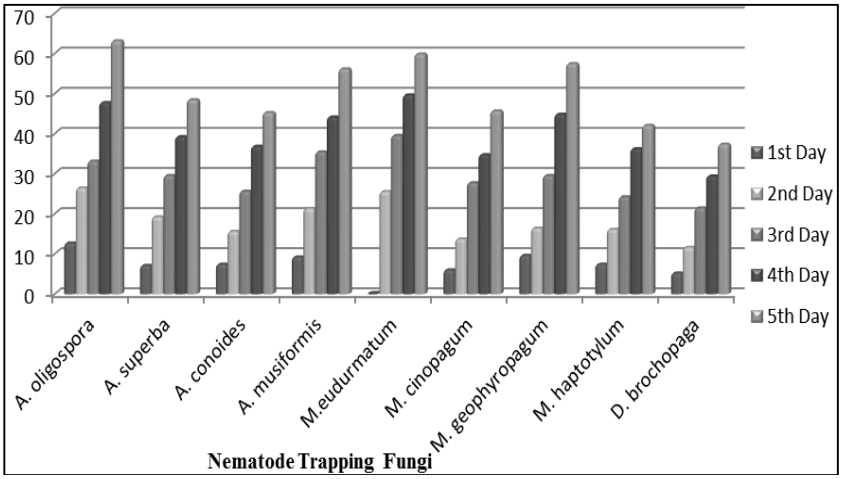


Fig 1: Temperature effect on growth of NTF at 20^{0}C

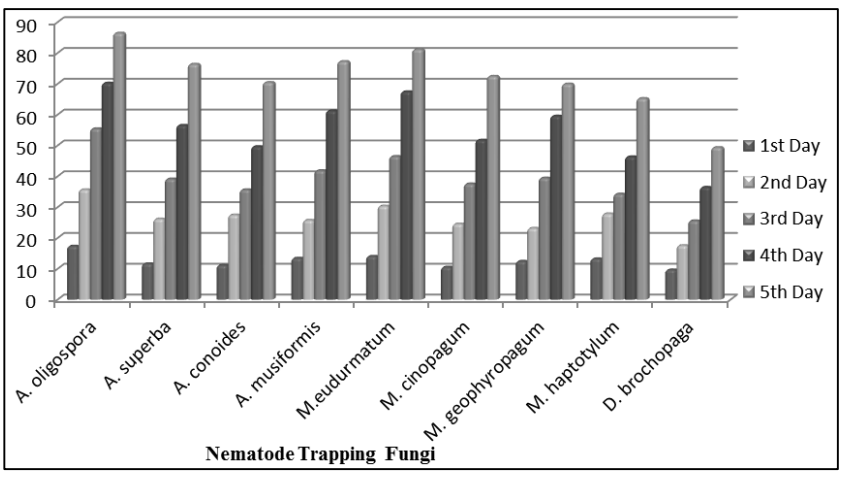


Fig 2: Temperature effect on growth of NTF at 25^{0}C

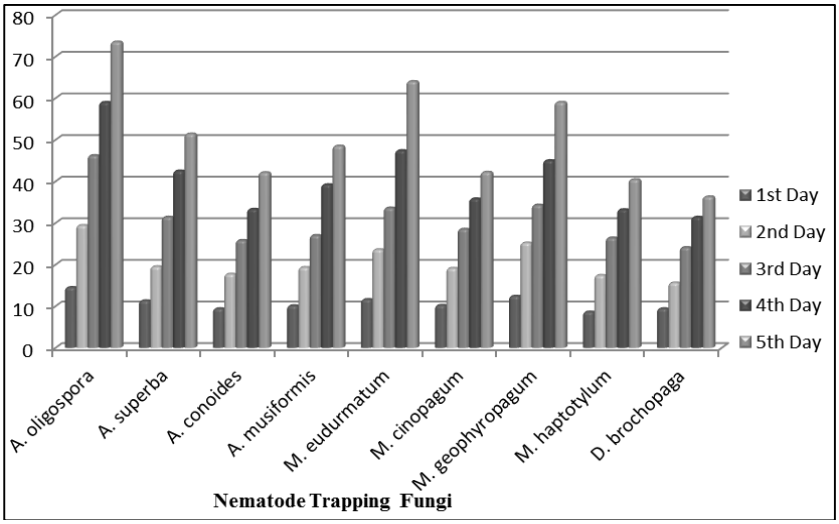


Fig 3: Temperature effect on growth of NTF at 30^0C

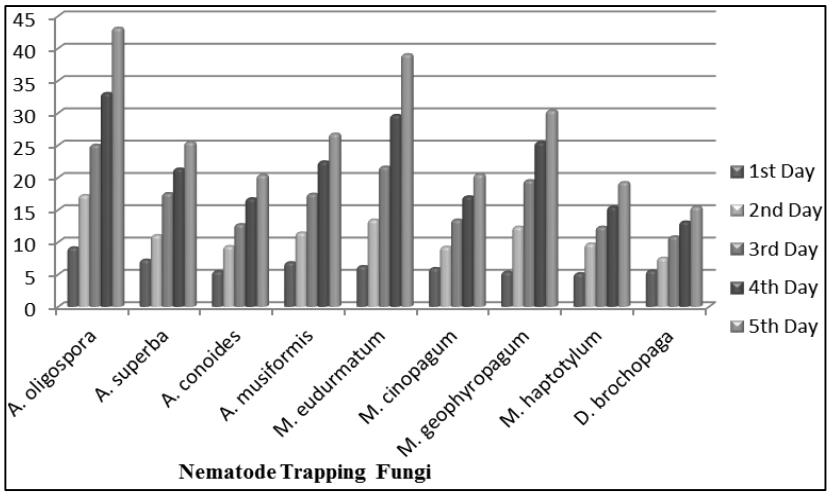


Fig 4: Temperature effect on growth of NTF at 35^0C

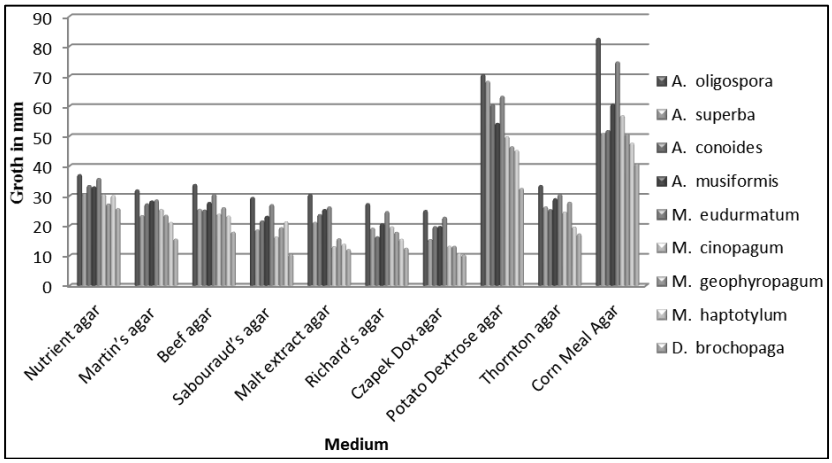


Fig 5: Effect of some media on the growth of Nematode Trapping Fungi at 25^0C after 5th Day

Effect of pH on growth and multiplication of nematode trapping fungi

Radial growth of nematode trapping fungi were tested at six different pH i.e. 5, 6, 7, 8, 9 and 10 on the corn meal agar medium and potato dextrose agar medium (fig.-6). The best suitable pH for the growth of nematode trapping fungi was recorded at pH i.e. pH-7 followed by at 8 and 6 pH respectively. The growth of nematode trapping fungi was recorded reading on both lower extreme and higher extreme of pH.

The maximum growth of *A. oligospora* was found in corn meal agar medium (fig.-6) at pH 7 as 70.8 mm followed by 64.3 mm and 31.2 mm at pH 8 and 6 pH respectively, on fifth day of observation. The lowest radial growth was observed in case of *A. oligospora* at 10 pH which were recorded 19.7 mm. While in case of *A. musiformis* the radial growth of 62.9 mm followed by 59.5 mm at 8.0 pH and 27.6 mm and 6 pH respectively. The minimum radial growth was recorded in *A. musiformis* at pH 10.0 as 19.1 mm. The radial growth of *D. brochopaga* on pH 7 was observed as 28.9 mm followed by

pH 8 as 26.3 mm. The radial growth were also recorded at 6 and 10 pH as 16.9 and 13.0 mm growth respectively after fifth day of inoculation.

The significant differences in growth and sporulation of nematode trapping fungi were found in *A. oligospora, A. musiformis* and *D. brochopaga* at pH 7, 8, 6 and the higher pH 10. The natural pH found good to growth and sporulation of *A. oligospora* compare to other nematode trapping fungi. At lower or higher pH then the natural pH it was observed reduction in growth rate of nematode trapping fungi. The 7 and 8 pH found good to growth and sporulation of nematode trapping fungi on corn meal agar medium.

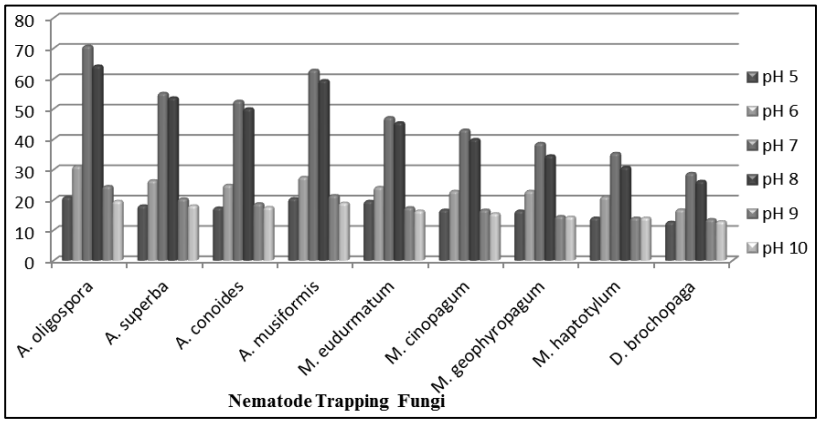


Fig 6: Effect of pH on corn meal agar for the growth of NTF at 25^0C after 5^{th} days

Effect of pH on growth and sporulation of nematode trapping fungi on Potato dextrose agar (PDA) medium

Growth and sporulation of nematode trapping fungi was assessed on different pH on potato dextrose agar medium at 25^0C (fig.-7). The growth was found maximum at 7 pH, in case of *A. oligospora* which was recorded as 67.5 mm followed by 54.2 mm at 8 pH. On the pH 6 and 10 pH, it was recorded reduction in growth rate at both the pH with the growth of 34.7 mm and 20.0 mm respectively after fifth day of inoculation. While in case of *A. musiformis*, the growth was recorded maximum as 60.9 mm followed by 49.7 mm at 7 and 8 pH. On the pH 6 growth rate was recorded 34.7 and the 18.9 mm growth was observed at 10 pH. The minimum growth was recorded in *D. brochopaga* as 25.3 mm at 7 pH which was at par with 24.6 at 8 pH. The 6 and 10 pH showed the growth of *D. brochopaga* as 17.4 mm and 15.8 mm after fifth day of inoculation.

In both the experiment, effect of pH on growth and sporulation of nematode trapping fungi on corn meal agar and potato dextrose Agar medium was conducted and found significant difference between different pH and both the medium. There was found no significant difference in growth at the pH 7.0 and 8.0. The corn meal agar medium was found best for growth and sporulation of nematode trapping fungi and the nematode trapping fungi *A. oligospora* found good ability to growth and sporulate on this medium compare to other nematode trapping fungi.

When growth and sporulation of nematode trapping fungi was assessed on corn meal agar media and Potato dextrose agar media at different pH, maximum growth of nematode trapping fungi was recorded at pH 7.0.

The result found to be similar by Nagesh *et al*. (2005) [11] who also reported that optimum ranged pH 6.0 and 7.5 was best for the growth and sporulation of *A. oligospora*. The pH has an indirect effect on growth of fungi. Variation in pH often leads to alteration of media components to yield products of greater or lesser toxicity (Mukhopadhyaya *et al.* (2001) [10]. The present study indicated that more acidic pH of 4 and 5 have deleterious effect on growth of both the isolates while no significant growth inhibition was observed between pH 6-8.

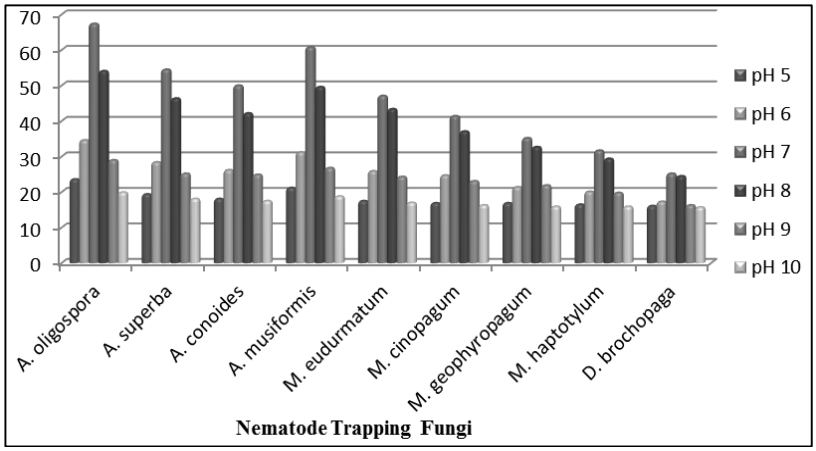


Fig 7: Effect of pH on potato dextrose agar for the growth of NTF at 25^0C after 5^{th} days

Effect of different Carbon and Nitrogen source utilization on nematode trapping fungi

Carbon utilization

Results of utilization of different carbon sources by these nematode trapping fungi summarized in Table 1. Among the carbon sources of sucrose and maltose supported appreciable growth of all the nematode trapping fungi with maximum growth of *A. oligospora* as 81.33 mycelial dry mass (MDM) on sucrose and 78.31 mycelial dry mass on Maltose. Dextrose supported less growth of *A. oligospora* as 66.47 mycelial dry

mass while the growth was better on the glucose 74.67 mycelial dry mass. *A. conoides* showed moderately good growth in sucrose 75.61 mycelial dry mass and maltose 71.16 mycelial dry mass. Less growth was observed in *D. brochopaga* on 34.15 mycelial dry mass better on dextrose. Utilization of carbon source by all the nine species was appreciable on sucrose as well as maltose as a carbon source.

Nitrogen utilization

Among the nitrogen utilization sources (Table- 2), sodium nitrate and ammonium sulphate both supported good growth, specially being effective in *A. oligospora* as 74.33 mycelial dry mass and 70.33 mycelial dry mass respectively. The less growth of *A. oligospora* was assessed on ammonium chloride as 66.28 mycelial dry mass. The utilization of nitrogen source by *A. conoides* showed moderately good growth on sodium nitrate as 69.26 mycelial dry mass and 67.09 mycelial dry mass on ammonium sulphate. Data showed that the poor growth was observed in *D. brochopaga* as 35.61 67.09 mycelial dry mass on ammonium chloride.

The main purpose of the present investigation was the determination of the nutritional requirements of nine nematode trapping fungi which capture nematodes by forming an adhesive network, sticky knob and constricting ring.

All the nine studied fungi were found to utilize a wide range of carbon sources. Based on our study sucrose, maltose, dextrose, and glucose were able to be utilized by *A. oligospora*, but sucrose was determined as a most effective carbon source for the growth of *A. oligospora.* Sucrose a disaccharide that is a molecule composed of two hexoses. In order to use sucrose, a fungus must separate the two hexoses with the enzyme invertase. *A. oligospora* possesses invertase probably, which makes possible to utilize sucrose as nutrient source. In addition, the ability to utilize a wide range of carbon sources for *A. oligospora* gave them a competitive advantage in a organic matter-rich soil, where the major limiting factor was nutrient competition with other soil microorganisms.

Satchuthananthavale and Cooke (1987) [16] concluded that network-forming species were efficient in utilizing all carbohydrates. It has been suggested that during the decomposition of organic matter in soil, these fungi were capable of trapping nematodes only when suitable carbon sources are available to provide energy for growth (Cooke 1982).

These network-forming species appear to be versatile in utilizing a wide range of compounds as a sole source of nitrogen. The nitrogen requirements of these fungi appear to be sufficiently fulfilled by inorganic sources. *A. oligospora* utilized several nitrogen sources and grew well especially when corn meal medium was supplemented with sodium nitrate. Utilization of sodium nitrate indicates that sufficient amount of enzyme reductase is present in A. *oligospora*. Sodium nitrate was known as the most readily assimilated nitrogen sources in most of the soil fungi (Shankar *et al.*, 1994, Saxena, 1989) [15, 18] studied two, nematode trapping fungi, *Monacrosporium cystosporum* and *A. conoides* and found they grow two times better in liquid medium with sodium nitrate than with ammonium nitrate as a nitrogen source

On the basis of present investigation and other published work, the network- forming species may be categorized as a group that is capable of saprophytic existence in soils, being able to utilize a wide range of carbon and nitrogen compounds. When subject to nutritional stress, they form traps and colonize nematodes. Thus environments deficient in nutrients are likely to stimulate the predatory phase while environments with nutrients in an available form are likely to be congenial for the saprophytic phase.

Table 1: Effect of different carbon sources on growth of NTF at 25^0C after 20^{th} days

NTF	Mean mycelial dry mass (mg/flask)			
	Sucrose	Dextrose	Maltose	Glucose
A. oligospora	81.33±0.46	66.47±0.82	78.31±0.69	74.67±0.44
A. superba	65.07±0.35	56.12±0.38	71.44±0.60	65.28±0.48
A. conoides	75.61±0.76	59.25±0.43	72.36±0.77	71.16±0.56
A. musiformis	61.92±0.89	51.34±0.79	65.21±0.37	61.85±0.81
M. Eudurmatum	72.37±0.52	53.19±0.32	68.10±0.31	68.33±0.58
M. cinopagum	61.18±0.24	47.25±0.41	60.29±0.92	56.17±0.38
M. geophyropagum	57.26±0.42	41.29±0.67	57.49±0.61	54.05±0.61
M. haptotylum	53.09±0.58	38.08±0.57	50.25±0.79	47.53±0.58
D. brochopaga	46.58±0.93	34.15±0.42	42.66±0.63	38.69±0.88
$p<0.05$	1.23	1.13	1.32	1.25

*DOI- Days of inoculation

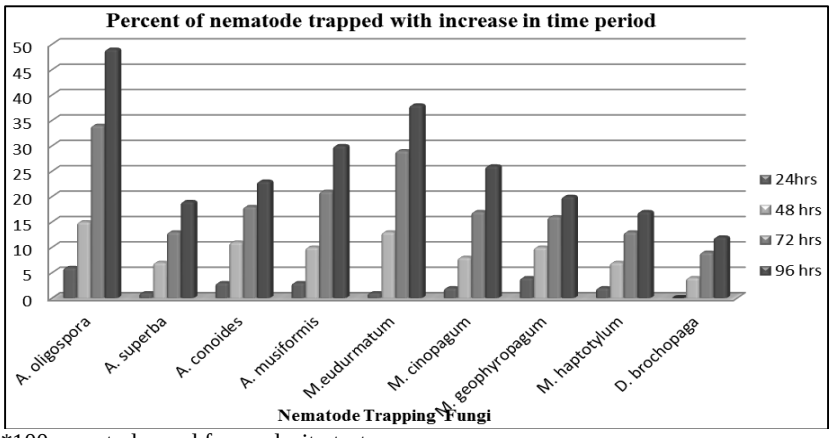
Table 2: Effect of different nitrogen sources on growth of NTF at 25^0C after 20^{th} Days

NTF	Mean mycelial dry mass (mg/flask)			
	Sodium nitrate	Potassium nitrate	Ammonium chloride	Ammonium sulphate
A. oligospora	74.33±0.73	70.21±0.43	66.28±0.67	70.33±0.66
A. superba	63.19±0.64	63.35±0.61	60.08±0.65	60.17±0.74
A. conoides	69.26±0.80	65.16±0.34	61.19±0.63	67.09±0.89
A. musiformis	60.09±0.62	58.42±0.44	56.33±0.63	60.23±0.77
M. Eudurmatum	65.25±0.64	65.39±0.71	60.25±0.79	62.39±0.79
M. cinopagum	60.15±0.48	50.14±0.50	46.39±0.73	55.12±0.44
M. geophyropagum	57.10±0.28	46.09±0.55	40.15±0.51	49.08±0.55
M. haptotylum	51.44±1.25	46.23±0.70	38.53±0.53	44.15±0.59
D. brochopaga	40.61±0.10	36.51±0.47	35.61±0.75	38.63±0.75
$p<0.05$	1.53	1.09	1.32	1.40

*DOI- Days of inoculation

Screening of nematode trapping fungi on the basis of predacity:

Observation on predacity of nematode trapping fungi against 2nd stage juvenile of *M. incognita* (fig.-8) showed that there was 49% killing of nematodes just after 96 hour when 100 nematodes were added to the petri plates of *A. oligospora* while it started increase in predacity as 6%, 15% and 34% after 24, 48 and 72 hrs after incorporation of nematodes respectively and in the case of *M. eudurmatum* 2nd stage juvenile of *M. incognita* was trapped as 38% after 96 hour of inoculation. The lowest percentage of predacity was observed in *D. brochopaga* with no predacity up to 24 hours and only 12% predacity was recorded even after 96 hours after addition of nematode.



*100 nematode used for predacity test

Fig 8: Screening of nematode trapping fungi on the basis of predacity

Screening of nematode trapping fungi on the basis of trapping structure

The *Arthrobotrys* spp., such as *A. oligospora, A. conoides, A. musiformis, A. superba* and *M. eudurmatum* all of which all form three-dimensional adhesive nets to trapped nematodes. The *Monacrosporium* spp., such as *M. cinopagum, M. geophyropagum* and *M. haptotylum* produces both Adhesive branches and adhesive knobs to trapped nematodes. The selected nematode trapping fungi are screened on the basis of trapping structure formed. *A. oligospora* formed the 2+ traps (=26-50) no. of traps in the presence of 100 nematode added to petri plates after 96 hours of incorporation. *A. oligospora* form three-dimensional adhesive nets which trapped nematodes and increasing trend of traps was observed with the increase of time period. *D. brochopaga* produced constricting rings to capture nematodes mechanically by the swelling of the ring cells. Initially after 24 hours of that 1+ traps were observed in the *A. oligospora, M. cinopagum, M. haptotylum* and *D. brochopaga*. The less number of traps as 1+ were observed in *A. conoides* and *A. superb* even after 16 hours. (Table-3)

Table 3: Screening of nematode trapping fungi on the basis of trapping structure

NTF	No. of trapping devices on different hours			
	24hrs	48 hrs.	72 hrs.	96 hrs.
A. oligospora	1+	1+	2+	2+
A. superba	0	0	1+	1+
A. conoides	0	0	0	1+
A. musiformis	0	1+	1+	2+
M. eudurmatum	0	2+	2+	2+
M. cinopagum	1+	2+	2+	2+
M. geophyropagum	0	1+	2+	2+
M. haptotylum	1+	1+	2+	2+
D. brochopaga	1+	2+	2+	2+

0 = No traps, 1+ = 1-25 traps, 2+ = 26- 50 traps, 3+ = 51- 100 traps, 4+ = › 100 traps

Predacity and trapping structure induced by nematode trapping fungi was tested against second stage juvenile of *M. incognita* with the population of 100 from 24 hrs to 96 hrs. It was found that there was less killing and less trapping structure induced in all the population tested for the nematode initially which was increased with increase in period of observation. Among *M. incognita* more percentage of killings was observed in the plate of *A. oligospora* with increase in number of population and time. In case of trapping structure induced by nematode trapping fungi this was also increases in number with increase in nematode population and time of incorporation. More number of traps were found in most of the fungi only two fungi showed less number of trapping structure.

Singh *et al.* (2007) [17] studied *in vitro* predacity tests of four fungi and revealed that *A. dactyloides* was most effective in capturing and killing J_2 of *M. graminicola* followed by *D. brochopaga* and *M. eudermatum* which was also proved when predacity of different plant parasitic nematodes *viz. Xiphinema index, Hoplolaimus, Tylenchorynchus vulgaris, Meloidogyne graminicola* and *Heterodera canjani* was tested with 25 nematode population, maximum percentage of killing was observed with *Heterodera cajani* followed by *Tylenchorynchus vulagaris* after 96 hrs of observation period.

Kumar and Singh (2011) [9] concluded that the vary among the isolates of same fungi and nematode species predacity for nematode species they studied the *in vitro* predacity of five isolates of *Arthrobotrys dactyloides* against *Meloidogyne incognita* (J_2), *Tylenchorhynchus brassicae* and *Hoplolaimus indicus*. They found that all isolates of *A. dactyloides* captured and killed *M. incognita* and *T. brassicae* but not *H. indicus*.

Nord B Hertz (1968) [12] observed that the living nematodes induced trap formation in *Arthrobotrys oligospora* more rapidly than by addition of morphogenetic peptides. In nematode-induced morphogenesis, excreted substances as speptides and amino acids were only partly responsible for the effect. Additional effects were due to volatile substances from nematodes or to direct contact between living nematodes and the hyphae. It was proposed that living nematodes act primarily through another mechanism than peptide-induced morphogenesis.

Conclusion

The growth and sporulation of nematode trapping fungi was

observed on corn meal agar media was tested at temperature 20, 25, 30 and 35 ^{0}C. Optimum temperature for the growth of nematode trapping fungi was found as 25^{0}C-30^{0}C. While the maximum growth was recorded in *Arthrobotrys oligospora* among ten different media were tested for screening media on the growth and sporulation of nematode trapping fungi. Corn Meal Agar (CMA) and Potato Dextrose Agar (PDA) media was found very well for the growth of nematode trapping fungi.

When the growth and sporulation of nematode trapping fungi was assessed at different pH on corn meal agar and potato dextrose agar medium at pH 7 followed by 6 and 8 was found good for both the growth and sporulation.

All the nine studied fungi were found to utilize a wide range of carbon and nitrogen sources. *A. oligospora* was found to utilize a wide range of carbon and nitrogen sources. Based on our study the carbon and nitrogen source, sucrose and sodium nitrate were able to be utilized by *A. oligospora* and sucrose and sodium nitrate was determined as a most effective carbon and nitrogen sources for the growth behavior of *A. oligospora* from other tested carbon and nitrogen sources. Utilization of sodium nitrate indicates the sufficient amount of enzyme reduction take place in *A. oligospora.*

Trapping structure and predacity when observed against second stage juvenile of *M. incognita* with the population of 100 nematodes from 24 hrs to 96 hrs. The killing of *M. incognita* was observed in the plate of *A. oligospora* after 96 hours increases with time period. Similarly trapping structure induced in nematode trapping fungi observed increasing with more nematode population and time period. Generally in all the tested fungi only two fungi showed less number of trapping structure.

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