Egypt. Acad. J. Biolog. Sci., 5(1): 115-126 (2012) **A. Entomology**
Email: egyptianacademic@yahoo.com **ISSN: 1687–8809**
Received: 17/5/2012 **www.eajbs.eg.net**

Effect of cultural condition on biomass production of some Nematophagous fungi as biological control agent

Mostafa Hasanzadeh*, Mehrnoush Mohammadifar, Nvazallh Sahebany, and Hasan Rreza Etebarian

Plant Protection Department, Abooreihan Campus, University of Tehran, Iran
*Email: mostafahasanzadeh14@gmail.com

ABSTRACT

Nematophagous fungi include a group of fungal antagonists to nematodes. The nematophagous fungi have been suggested as promising candidates for biological control of plant parasitic nematodes. Impacts of different pH levels, temperature, light intensity, carbon and nitrogen source were tested on the growth of five nematophagus fungi under in vitro conditions. Three isolates of *Trichoderma harzianum* T7, T8, and T14, one isolate of *Pochonia chlamydosporia var. chlamydosporia* and one isolate of *Arthrobotrys oligospora* were tested. The results of experiment indicated that the growth of isolates of *Trichoderma harzianum* was maximum in pH range of 7.00-8.00. The best growth of *Pochonia chlamydosporia var. chlamydosporia* and *Arthrobotrys oligospora* was in 4.00-7.00. Effect of temperature differs between range of 15-30°C, for isolates of *Trichoderma harzianum* was 20-25°C, *Pochonia chlamydosporia var. chlamydosporia* was 15-20°C, and *Arthrobotrys oligospora* was 20-25°C. Maximum growth of two isolates of *Trichoderma harzianum* T7, T8 and *Pochonia chlamydosporia var. chlamydosporia* was in darkness. Best growth of one isolates of *T. harzianum* (T7) was in light and maximum growth of *Arthrobotrys oligospora* and *Trichoderma harzianum* T14 occurred in 12 h light and 12h dark. The best source of nitrogen for *Trichoderma harzianum* (T8,T14) was Nitrate potassium, while Nitrate ammonium was the best nitrogen source for (T7).The maximum growth of *A. oligospora* and *P. chlamydosporia* occuerd in Nitrate sodium. Between carbon sources Arabinose are suitable for *A.oligospora* and *Trichoderma harzianum* (T7).The maximum growth of *P. chlamydosporia* and *T. harzianim* (T14) occuerd in the use of fructose. Best growth of *T. harzianum* (T8) was in Mnitole.

Keywords: *Arthrobotris oligospora,Trichoderma spp*. ,*pochonia chlamydosporia* ,PH ,Tempercher, Light,Carbon,Nitrogen

INTRODUCTION

Nematodes one of abundant and successful organisms which are located in the very nest of ecological (Xingzhong *et al.* 2009). They are parasites of plants and animals on earth. Nematode effects on humans are by reducing of crops, direct infection of humans and incapable of domestic animals. More than a billion people worldwide are infected to nematodes, and nematodes are responsible for exotic

diseases such as elephantiasis. Plant parasitic nematodes are probably the only uncontrollable organisms that cause damage and stress in plants. Nematode infection has a direct impact on the roots so that the roots are reduced in a few days (Arntzen *et al.*1994; Rawsthorne and Hague, 1986). The use of natural enemies of plant-parasite nematodes as biological control organisms of the nematode diseases of crop plants is certainly not a recent idea to Plant patologist. Cobb (1917), the forerunner of nematology in the US, proposed that predacious nematodes can be used as biological control of plant parasitic nematodes. Anyway nematologists such as Mankau (1980), Kerry (1980) and Stirling (1984), as well as others, have summarized investigations on the many soil microorganisms as management tools of several plant parasitic nematode diseases. Nematophagous fungi are carnivorous fungal species that use their spores or mycelial structures to capture vermiform nematodes, or use their hyphal tips to parasitize the eggs and cysts of nematodes (Nordbring Hertz 2004), or produce toxins to attack nematodes (Li *et al*. 2000). They are the natural enemies of nematodes and have developed very sophisticated strategies to either infect or capture these small animals. Nematophagous fungi are a diverse group of microorganisms, and their nematophagous habit is generally considered to have evolved independently in different fungal classes. Nematophagous fungi or fungi destructive to nematodes can be infect, kill and digest nematodes in each of three phses (eggs, larvae and adults). This soil fungus is present in most parts of the world and is found in all types of weather (Barron 1977). Many of the nematophagous fungi are facultative parasites and they can survive in soil as a saprophyte. If there is a host plant, they can change from a saprophytic to a parasitic stage and produce a infection form structures, e.g. trapping organs, hyphal coils or appressoria. These infection structures vary depending on the type of host nematode, fungus or plant. Nematophagous fungi from three groups were formed; endoparasites, predatory and ovicidal. They are worldly fungi take place in natural and agricultural soils and in all types of decomposing organic matter (Mota *et al.* 2003). The ovicidal group is a parasites of eggs and nematodes, but in the absence of host plants will survive as a saprophyte (Lysek *et al*. 1982). The eggs of the nematode parasite fungi can affect the development of eggs in the soil and cause their ruining. Studies on natural processes of destroying nematodes eggs by this fungus in the early stages, but they represent an alternative approach which, if used with other prophylactic measures, may help control species of importance (Lysek *et al.* 1982; Araujo *et al*. 2009). The use of these fungi to parasite the egg of nematodes, is an important biological phenomenon, that can be used for biological control of plant parasitic nematodes. Between the favorable species from this group, the fungus *Pochonia chlamydosporia*, a deuteromycete, and facultative parasite. This fungus is greatly distributed and has been successfully used in laboratory conditions for the parasite of eggs of Meloidogyne sp. (Kerry and Hidalgo 2004), Ascaris suum (Araújo *et al.* 2008).These fungi through the production of appressorium from non specialized hyphae surround the egg surface and with chemical and physical methods penetrate the egg. (Lysek and Sterba.1991; Sharon *et al*. 2001) reported reduced root galling on tomato by *Meloidogyne javanica* (Tylenchida:Meloidogynidae) after soil pre plant treatment with a peat bran

preparation of *Trichoderma harzianum*. The nematode trapping fungus *Arthrobotrys oligospora* captures nematodes by using special hyphae which forms a three dimensional networks (Anders *et al.* 1991). *Arthrobotrys oligospora*, has shown the highest potential to control the multiplication of *Meloidogyne mayaguensis*, a root knot nematode parasitizing those plant species or cultivars resistant to other tropical Meloidogyne species (Duponnois *et al.* 1995). However, developing some fungi as potential biopesticides is difficult because their mycelial growth on artificial media usually depends on the fungal species and on the components used in the culture media (Latge´ & Sanglier 1985). The nutritional requirements for fungal growth vary among different biocontrol agents, for example, nematophagous fungi are greatly influenced by nutrients and culture conditions (Bricklebank & Cooke 1969; Saxena *et al*. 1989; Li & Holdom 1995).

Therefore, the objective of this experiment was to study physiological and nutritional requirements of different isolates of nematophagus fungi in order to understand differences among the isolates for the proper mass production of this fungi.

MATERIALS AND METHODS

Fungal inoculum preparation

All fungi were obtained from plant pathology department, agriculture faculty of Tehran University and were cultured on potato dextrose agar (PDA, Oxoid). Seven days after incubation (25°C), the purified fungi were used in experiment.

Physiological study

Effect of temperature

The fungi were subjected to different temperature conditions to study the best suited temperature level for the growth of the fungus. Potato Dextrose Broth medium was used in one experiment to study the growth in liquid medium. Thirty milliliter of liquid medium was poured into a 150ml conical flask under aseptic conditions and inoculated with 5 mm diameter identical culture discs of each isolate. The experiment was done by four replicates. Inoculated conical flasks containing PDB medium were incubated at 10±1°C,15±1°C, 20±1°C, 25±1°C and 30±1°C. Dry mycelial weight was recorded in the liquid cultures ten days after the incubation.

Effect of pH

Effect of pH on the growth of those isolates was also tested in the laboratory using liquid cultures containing different pH levels. Potato Dextrose Broth medium was used to study the effect of pH of medium on the growth of different isolates of nematophagus fungi. Thirty milliliter of liquid medium was poured into a 150 ml conical flask under aseptic conditions. The Reaction of the medium was adjusted to the desired pH by adding 0.1N NaOH or 0.1N HCl (Naik *et al*. 1988). The medium was buffered with Disodium hydrogen phosphate citric acid buffer according to the schedule of Vogel (Vogel 1951). Flasks were sterilized at 121 0C at 15 psi for 20 minutes. Each flask was inoculated with each isolate using 5 mm diameter mycelial disc in sterile conditions. Inoculated flasks were incubated at 25±1°C for ten days and

the dry mycelial weights were obtained. The cultures were filtered through whatman No. 42 filter paper and the dry mycelial weight was measured by subtracting the initial weight of the filter paper from the weight of the filter paper along with the mycelial mat.

Effect of Light Regime

The fungi cultures of different isolates on PDB were exposed to continuous light, dark and 12h light and 12h darkness in an environment chamber maintained at 25±1°C. Mycelial disc of five mm of each isolate was used to inoculate conical flask. Four replications were maintained for each treatment. Inoculated flasks were kept in environment chamber and light intensity was adjusted to required level. The mycelia growth was measured in each case ten days after inoculation.

Nutritional study

The utilization of carbon and nitrogen nutrition was studied by replacing the fructose and potassium nitrate in the basal medium with various nitrogen and carbon compounds on the molecular weight basis. Potato broth was used as a basal medium for studying carbon and nitrogen. Thirty milliliters of the medium dispensed in 150 ml conical flasks were sterilized and used for inoculation with the fungus. All the flasks were inoculated with 5 mm diameter mycelial disk obtained from 7 day old single spore cultures of nemaophagus fungi isolates and incubated at 25±10C for ten days. The cultures were filtered through whatman No. 42 filter paper and the dry mycelial weight was measured by subtracting the initial weight of the filter paper from the weight of the filter paper along with the mycelial mat.

Effect of carbon source

Carbon compounds tested in the study were Sorbitol, Arabinose, Fructose and Manitol. Potato broth medium without adding fructose was used as a control. Potassium nitrate was used as a source of nitrogen for all treatments. Carbon sources were added to the basal medium (potato broth medium) at 21.053g of carbon per liter of medium. Each flask containing different carbon sources was inoculated with a 5 mm mycelial disk of seven day old fungal cultures and incubated for ten days.

Effect of nitrogen source

Four different nitrogen sources used in this study were Ammonium nitrate (NH_4NO_3), Potassium nitrate (KNO_3), Sodium nitrate ($NaNO_3$) and L-Proline. Different nitrogen sources were added into potato broth medium at 1.3855g of nitrogen per liter of the medium. Fructose was used as the source of carbon in all the treatments. Potato broth without adding Potassium nitrate (nitrogen source) was used as the control. All flasks were inoculated with 5 mm mycelial disks of seven day old fungal culture under aseptic condition and incubated for ten days.

Evaluation the experiment: growth of fungi in all experiments was measured. All experiments were arranged with 4 replicates in a completely randomized design (CRD). Experimental data was statistically analyzed using SAS software (ANOVA) and means were separated by Duncan’s multiple range test (P=0.05).

RESULTS

Effect of temperature

Temperature differences among all isolates were significant (P=0.05). Different isolates of fungi responded differently to various temperature regimes as shown in the Table1 and Fig.1. Mean colony biomass of isolates on liquid medium, was maximum at 20-25°C. Growth of three isolates of *Trichoderma harzianum* and *Arthrobotrys oligospora* was maximum at 20-25°C. *Pochonia chlamydosporia var. chlamydosporia* had better growth on15-20°C. There were significant interactions between isolates and temperature levels. Temperature of 10-15°C found to be not suitable for the growth of those isolates. Among the external factors which influence the growth of fungi, temperature plays an extremely important role.

Table 1: Growth of different isolates Nematophagus fungi in different temperature in PDB media

Mean mycelial weight (mg/30ml)*					
Isolate	10°C	15°C	20°C	25°C	30°C
T7	54.5^{c}	110.5^{b}	113.25^{b}	132.25^{a}	110.25^{b}
T8	62.75^{c}	152.5^{a}	149.5^{ab}	155^{a}	140.5^{b}
T14	51^{c}	174.75^{a}	132.75^{b}	149.25^{b}	135.75^{b}
A.oligospora	48.75^{c}	106^{b}	142.5^{a}	131.75^{a}	99.25^{b}
P.chlamydosporia	93^{b}	94.5^{b}	132.75^{a}	40.75^{c}	60.75^{c}
Mean	62.16	125.4	129.85	129.2	110

*Values are means of four replicates**.**

Values within a row followed by a same letter are not significantly different at *P*=0.05 according to Duncan's multiple range test.

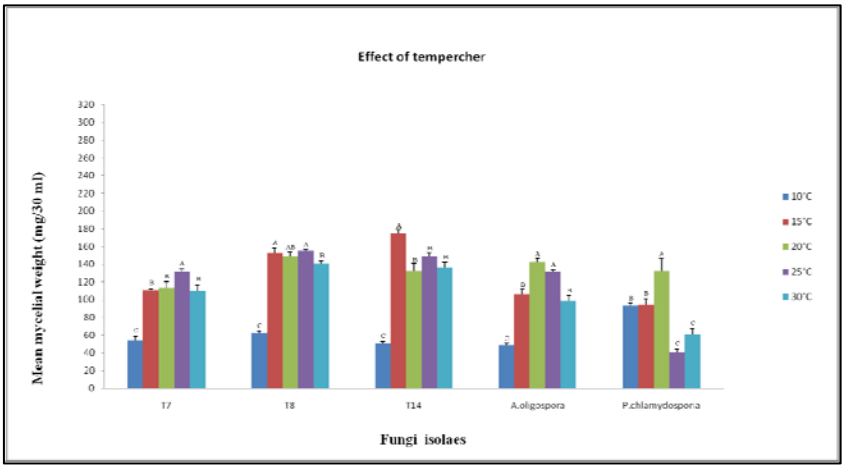

Fig. 1: Effect of temperature on the biomss production of Nematophagus fungi.

Effect of pH

The mycelial growth was different among isolates and different pH levels (P=0.05). The interaction between isolates and pH levels was also significant (P=0.05). At all pH levels tested, *Trichoderma* isolates (T7, T8) grew significantly better in 7-8 than the other level. In *A. oligospora* recorded maximum growth at pH 5-6 while pH 4-5 supported the maximum growth of *P.chlamydosporia*. In Trichoderma harzianum (T14) best growth occurred in PH 6-7 (Table 2 and Fig.2).

Table 2: Growth of different isolates of Nematophagus fungi in different PH in PDB media.

Mean mycelial weight (mg/30ml)*					
Isolate	4	5	6	7	8
T7	83.25^{c}	83.75^{c}	81.75^{c}	115.25^{b}	136.25^{a}
T8	54^{c}	57.25^{c}	58^{c}	81.75^{b}	110.25^{a}
T14	113.5^{b}	115.5^{b}	119.5^{b}	143.25^{a}	44.5^{c}
A.oligospora	131^{a}	110.5^{b}	108.25^{b}	110.25^{b}	94^{b}
P.chlamydosporia	169.25^{a}	161.25^{a}	156.75a	149.75^{a}	101.25^{b}
Mean	101	106	104.5	115.6	100.3

*****Values are means of four replicates.

Values within a column or row followed by a same letter are not significantly different at P=0.05 according to Duncan's multiple range test.

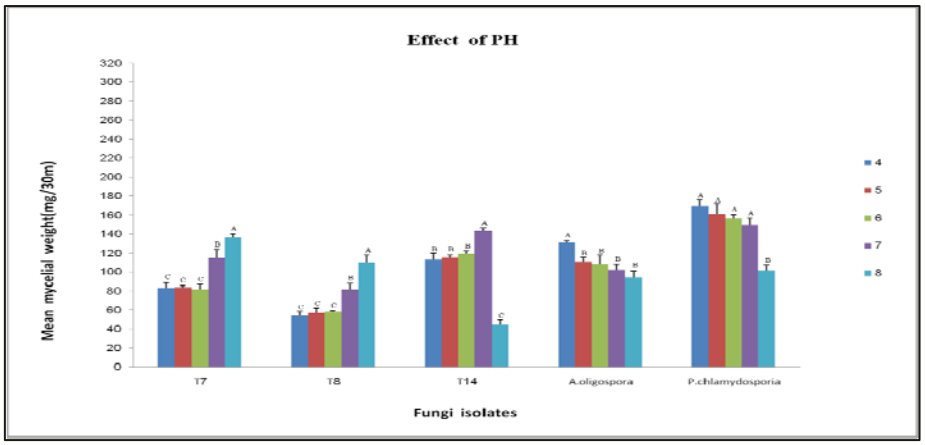

Fig. 2: Effect of PH on the biomss production of Nematophagus fungi.

Effect of light intensity

Light has profound effect on the mycelial growth of nematophagus fungi. The exposure of the fungus to alternate cycles of 12 h light and 12 h darkness and darkness for 10 days resulted in the maximum mycelial growth of Trichoderma harzianum T14 which was significantly superior over other two treatments tested (Table 3 and Fig.3). The maximum growth of *A. oligosporawas* one that exposed to continuous light resulted and *Trichoderma harzianum* T8 and *P. chlamydosporia* gerw best in, continuous darkness (Table 3).

Table 3: Growth of different isolates of Nematophagus fungi in different light intensity in PDB media.

Mean mycelial weight (mg/30ml)*			
Isolate	light	dark	12 h alternate dark and light
T7	100^{a}	78.5^{b}	102.5a
T8	99.75^{b}	110.75^{a}	113^{a}
T14	103^{b}	125.25^{a}	125.25^{a}
A.oligospora	146.5^{a}	98.5^{b}	138^{a}
P.chlamydosporia	139.75^{b}	164^{a}	174.25^{a}
Mean	101	106	104.5

*****Values are means of four replicates.

Values within a column or row followed by a same letter are not significantly different at P=0.05 according to Duncan's multiple range test.

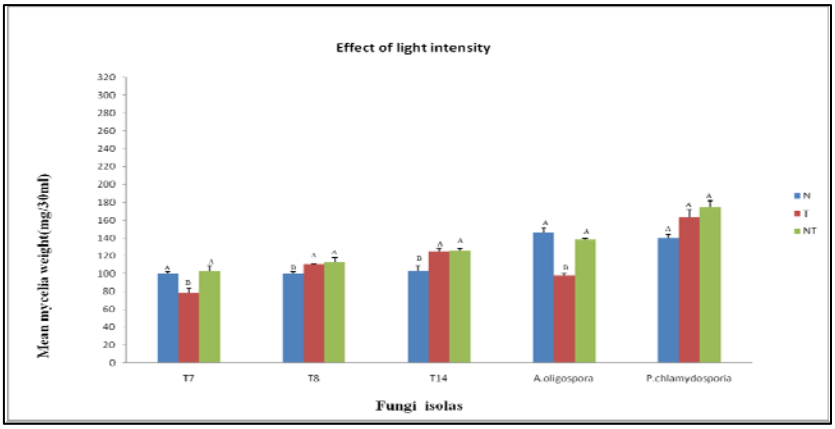

Fig. 3: Effect of light intensity on the biomss production of Nematophagus fungi.

Effect of carbon source

The growth of the fungi isolates was different among isolates (P=0.05) and among different carbon sources (P=0.05). Sorbitol was the least utilized carbon compound by Trichoderma isolates (Table 4 and Fig.4). The fungi grown on Arabinose and Fructose recorded significantly higher dry mycelial weight followed by manitol. *Arthrobotris oligospora* had significantly higher mycelial growth on sorbitol followed by Arabinose, while in manitol the least mycelial growth occurred. The interaction between isolates x carbon sources was also significant. There was minimum growth observed when those isolates were grown on a medium without any carbon source (control).

Table 4: Growth of different isolates Nematophagus fungi in different carbon source in PDB media

	Mean mycelial weight (mg/30ml)*				
Isolate	Control	Sorbitol	Manitol	Froctose	Arabinose
T7	23^{d}	72.75^{c}	145^{b}	151.5^{b}	184^{a}
T8	19.75^{d}	47^{c}	147.25^{a}	123^{b}	112.75^{b}
T14	33.25^{d}	100^{c}	160.75ab	174.5^{a}	157.5^{b}
A.oligospora	166.75^{b}	215.5^{a}	51.5^{d}	122.5^{c}	223.25a
P.chlamydosporia	53^{e}	235^{b}	188.75^{d}	278^{a}	213.25^{c}
Mean	62.16	125.4	129.85	129.2	110

*Values are means of four replicates**.**

Values within a row followed by a same letter are not significantly different at *P*=0.05 according to Duncan's multiple range test.

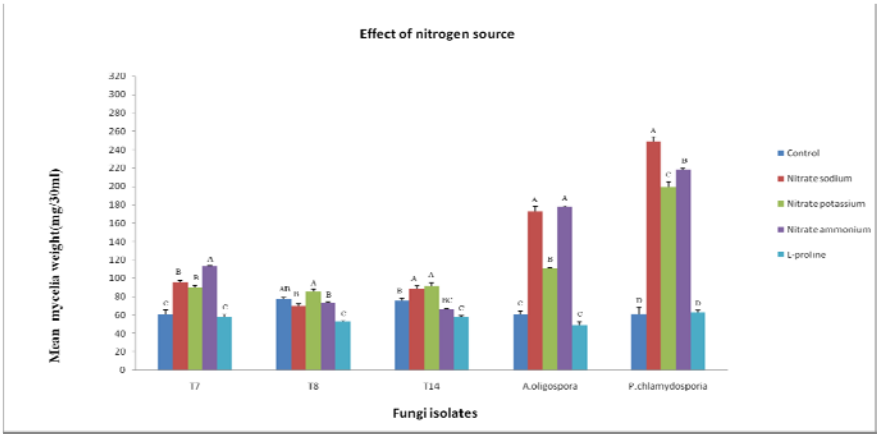

Fig. 4: Effect of Nitrogen source on the biomss production of Nematophagus fungi.

Effect of Nitrogen source

Mycelial growth of the fungus was influenced by all the nitrogen sources and was statistically on par with the control, which had no nitrogen source (Table 5 and Fig 5). Nitrate amonium supported the maximum growth followed by sodium nitrate and Nitrate potassium. L-prolin was the least efficiently utilized nitrogen source by all fungi isolates. Utilization of nitrogen sources differs significantly among different isolates as presented in the (Table 2). Growth of these fungi on different nitrogen sources was significantly higher than the rest of the isolates.

Table 5: Growth of different isolates Nematophagus fungi in different nitrogen source in PDB media.

Mean mycelial weight (mg/30ml)*					
Isolate	Control	Nitrate sodium	Nitrate potassium	Nitrate ammonium	L-proline
T7	61^{c}	95.5^{b}	90^{b}	113^{a}	57.75^{c}
T8	77^{ab}	69.75^{b}	85^{a}	73.75^{b}	53^{c}
T14	75.5^{b}	88.5^{a}	92.25^{a}	66.25^{ba}	57.75^{c}
A.oligospora	61^{c}	172.5^{a}	110.5^{b}	177.75^{a}	49.25^{c}
P.chlamydosporia	61.5^{d}	249.25^{a}	199.25^{c}	218.5^{b}	63^{d}
Mean	62.16	125.4	129.85	129.2	110

*Values are means of four replicates**.**

Values within a row followed by a same letter are not significantly different at *P*=0.05 according to Duncan's multiple range test.

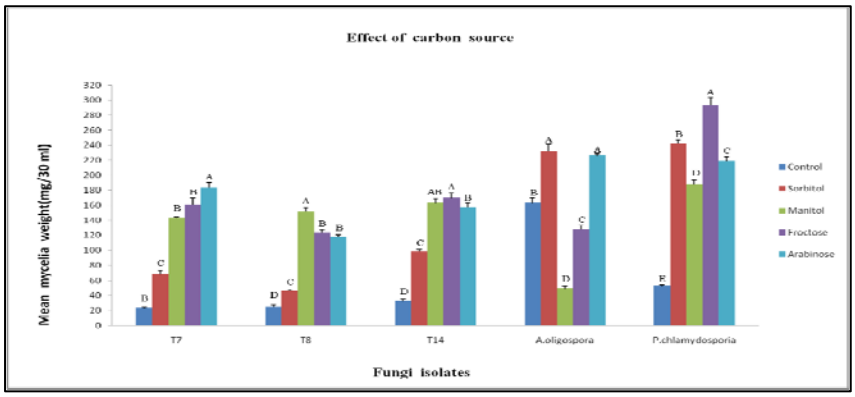

Fig. 5: Effect of Carbon source on the biomss production of Nematophagus fungi.

DISCUSSION

Temperature affects almost every function of the fungi (Lilly and Barnett 1951). Papavizas (1985) has stated that different species of *Trichoderma* have their own ecological preferences. However, the species of *Trichoderma* are distributed worldwide. Most of the *Trichoderma* strains are mesophilic. Low temperatures in winter may cause a problem during biological control by influencing the activity of the biocontrol agents. Studies are available on the effects of temperature on the spore germination and germtube growth (Magan *et al*.1988), mycelial growth (Danielson *et al*. 1973, Samuels 1996), competitive saprophytic abilities (Naar *et al*. 1998; Badham 1991, Eastborn *et al*. 1991) and on volatile and non volatile metabolite production (Tronsmo *et al*. 1978) of *Trichoderma* strains. Most Trichoderma strains are mesophilic, and cannot protect germinating seeds from soilborne diseases caused by

cold tolerant strains of plant pathogenic fungi during cold autumn and spring conditions. The mycelial growth and sporulation of *P. chlamydosporia* are influenced by components of the medium and culture conditions (Kerry *et al*.1986; Zaki *et al.* 1993) (Nagesh *et al*. 2007) report that best temperature for growth of *P. chlamydosporia* was 25-35^{o}C. (Arevalo *et al*. 2009) report that the optimum temperature for growth and spore production ranged between 24 and 28°C for *P. chlamydosporia* isolates. (Fernandes *et al*. 199) all fungal isolates grew faster at a constant 20°C on Potato Dextrose Broth. (Duponnois *et al*. 1995) record that optimum growth of *A. oligospora* occurred at 25-30°C. (Pandey 1973; Sanial 2000) demonstrate that best temperature for growth of *A. oligospora* was 25°C. (Morgan et al. 1997) record that peak growth rate was found to occur between 20 and 25 °C for *A. oligospora*. (Nagesh *et al*. 2005) demonstrate that the optimum temperatures for *A. oligospora* growth, sporulation, conidiospore germination and conidiospore production ranged were between 25 and 35°C. (Gomez *et al.* 2003) reported that two Cuban isolates of *Arthrobotrys oligospora* showed greatest and least growth at 25 and 32°C, respectively. (Uponnois *et al* .1995) demonstrate that the best PH for growth of *A.oligospora* was 5-6. (Nagesh *et al*. 2005) report that pH optima ranged between 6.0 and 7.5 for *A. oligospora*. (Nagesh *et al.* 2007) record that the optimum PH for *P. chlamydosporia* was 6.5-7.7 (Kredics *et al*. 2003) demonstrate that isolate of *Trichoderma* can grow in PH between 2-6 with an optimum at 4.0. Jackson *et al.* (1991) have found that optimum biomass production of three *Trichoderma* isolates occurred at pH ranges between 4.6 and 6.8. (Seyis *et al.* 2005) observed that the activity of *Trichoderma harzianum* was maximum around pH 5. It has been demonstrated that Trichoderma strains are active under a wider range of pH (Kredics *et al.*, 2003). (Duponnois *et al.* 1995) *A.oligospora* grew better on an acidic medium (pH 5.6) than on a basic one (pH 7.8). (Ming *et al.* 2005). For mycelial growth of P.chlamydosporia, the optimal pH range was 5-6; with pH 6.0 giving the greatest biomass. *Trichoderma spp* showed an ability to use a variety of carbon as well as organic and inorganic nitrogen compounds as sole source of carbon or nitrogen. Papavizas (1985) has stated that different species of *Trichoderma spp* have their own ecological preferences. However, the species of Trichoderma are distributed worldwide. From our study it is evident that the wide occurrence of *trichoderma spp* is supported by the fact that it has an ability to utilize a variety of nutritional factors as well as they have a broad range of pH and temperature tolerance for their growth and sporulation. Although with certain conditions the growth and sporulation were reduced, but *Trichoderma spp* was still able to grow. These observations are interesting for the ecological behavior of this antagonistic fungus. (Upadhyay and Rai, 1978, 1979) earlier reported that *Trichoderma spp*. prefer and grow well in the soils having acidic pH and high organic matter (Upadhyay and Rai, 1978, 1979). Our result shows that isolates of Trichoderma don’t have necessary need to nitrogen source, so that the amount of biomass produced in the control condition was not much different from the other nitrogen sources. But in other fungi, there was significant difference between the use of nitrogen and control.

CONCLUSION

It is widely known that environmental parameters such as abiotic (soil type, soil temperature, soil pH, water potential and such like) and biotic (plant species and variety, microbial activity of the soil) factors as well as other factors such as method and timing of applications may have influence on the biological control efficacy of fungi isolates of nematofagous fungi under study varied in their ability to grow in different temperature, light, pH levels ,nitrogen and carbon source. Most of the isolates preferred temperature range from 15 to 25 0C for the growth and isolates grew well at pH of 5 to 7.

REFRENCE

Araujo, J.M.; Araújo, J.V.; Braga, F.; Carvalho, R.O.; Silva, A.R. and Campos, A.K. (2000). Interaction and ovicidal activity of nematophagous fungus *Pochonia chlamydosporia* on *Taenia saginata* eggs. Experimental Parasitology, 121: 338-341.

Arevalo, J.; Hidalgo, D. L.; Martins, I.; Janaína, F.; Souza., Mauro, C. J., Castro., Maria, D. R.G., Carneiro. and Myrian, S., Tigano. (2009). Cultural and morphological characterization of *Pochonia chlamydosporia* and *Lecanicillium psalliotae* isolated from *Meloidogyne mayaguensis* eggs in Brazil. Tropical Plant Pathology, 34(3):158-163.

Arntzen, F.K., Visser, J.H.M and Hoogendoorn, J. (1994). The effect of the potato cyst nematode *Globodera pallida* on in vitro root growth of potato genotypes, differing in tolerance. Ann. Appl. Biol 124: 59–64.

Barron, G.L .(1977). The nematode destroying fungi. Topics in mycobiology No. 1 Canadian Biological Publications, Guelph.

Blaxter, M., Bird, D.(1997). Parasitic nematodes. In: Riddle DL, Blumenthal T, Meyer BJ, Priess JR, editors. *C. elegans* II. Cold Spring Harbor: Cold Spring Harbor Laboratory Press., pp. 851–78.

Boucher, G., Lambshead, P.J.D.(1994).Ecological biodiversity of marine nematodes in samples from temperate, tropical, and deepsea regions. Conservation Biology., 9:1594–604.

Bricklebank, J. and Cooke, R. C. (1969). Utilization of polysaccharides by two nematode parasitic fungi. Transactions of the British Mycological Society 52: 347–349.

Cobb, N. (1917). The mononchs, a genus of freeliving predatory nematodes. Contributions to a Science of Nematology. Soil Science 3: 431-486.

Duponnois, R., Mateille, T. and Gueye, M. (1995) .Biological characteristics and effects of two strains of *Arthrobotrys oligospora* from Senegal on Meloidogyne species (with reference to M. mayaguensis) parasitizing tomato plants. Biocontrol Sci Technol 5:517–525.

Goddard. (1986). Factors affecting growth in vitro. Nematologica; 32: 461–473.

Gomez, L., Baro, G., Sanchez, L., Rodriguez, M.G.(2003) .Identification and characterization of Cuban isolates of nematode trapping fungi. Revista de

Proteccion Vegetal, 18: 53-57.

Jackson, A.M., Whipps, J. M., Lynch, J. M. (1991). World J Microbiol. Biotech., 7: 494-501.

Kerry,B.R. (1980) .Progress in use of biological agents for control of nematodes. In: Behsville Symposia in Agricultural Research No. 5: Biological Control in Crop Production, pp. 79- 90.

Kerry, B.R.; Irving, F. and Hornsey, J.C. (1998). Variation between strains of the nematodphagous fungus, *Verticillium chlamydosporium*. Biol Contr; 11: 217–223.

Kevei, F., Nagy, E. (2003). Influence of Environmental Parameters on *Trichoderma* Strains with Biocontrol Potential. Food Technol. Biotechnol., 41 (1) 37- 42.

Kredics, L.; Antal, Z.; Manczinger, L. and Szekeres, A. (2003). Influence of Environmental Parameters on *Trichoderma* Strains with Biocontrol Potential Food Technol. Biotechnol., 41 (1) 37- 42.

Latge, J.P. and Sanglier, J. J.(1985).Optimisation de la croissance et de la sporulation de Conidiobolus obscurus en milieu de´fini. Canadian J. Botany 63: 68–85.

Li, D.P. and Holdom, D.G. (1995) .Effects of nutrients on colony formation, growth, and sporulation of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). J. Invertebrate Pathology, 65: 253–260.

Li, T.F.; Zhang, K.Q. and Liu, X.Z. (2000). Taxonomy of nematophagous fungi. Chinese Science and Technical Publishing, Beijing

Lilly, V.G. and Barnett, HL. (1951). Physiology of Fungi.. McGraw Hill Book Company Inc., New York, 464p .

Liu,X.; Meichun,X. and Yongsheng, C. (2009). The living strategy of nematophagous fungi. Mycoscience 50:20–25.

Lysek, H.; Fassatiová, O.; Pineda, N.C. and Hernández, N.L. (1980). Ovicidal fungi in soils of Cuba. Folia Parasitologica, 29:265–270.

Lysek, H. and Sterba, J. (1991). Colonization of Ascaris lumbricoides eggs by the fungus *Verticillium chlamydosporium* Goddard. Folia Parasitologica, 38: 255–259.

Manjunatha, R. R. and Rawal, R.D. (2002). Effect of carbon and nitrogen sources for growth and sporulation of *Colletotrichum gloeosporioides* and *Gloeosporium ampelophagum* causing grapevine anthracnose.Annu. meeting and symposium on plant disease scenario in southern India, Dec. 19-21, IPS (S Zone), UAS, Bangalore, 49pp.

Mankau, R. (1980). Biological control of nematode pests by natural enemies. Annual Review of Phytopathology 18: 415-440.

Minghe, M.; ChuanKun, X. and KeQin, Z. (2005). Effects of carbon and nitrogen sources, carbon-to-nitrogen ratio, and initial pH on the growth of nematophagous fungus *Pochonia chlamydosporia* in liquid culture. Mycopathologia 159: 381–387.

Morgan, M.; Behnke, J.M.; Lucas, J.A. and Peberdy, J.F .(1997). In vitro assessment of the influence of nutrition, temperature and larval density on trapping of the infective larvae of *Heligmosomoides polygyrus* by *Arthrobotrys oligospora*, *Duddingtonia flagrans* and *Monacrosporium megalosporum*. Sep., 115(Pt): 303-10.

Mota, M.A.; Campos, A.K. and Araújo, J.V .(2003) .Controle biológico de helmintos

parasitos de animais: estágio atuale perspectivas futuras. Pesquisa Veterinária Brasileira, 23:93–100.

Nagesh, M.; Hussaini, S.S.; Chidanandaswamy, B.S. and Biswas,S. R. (2005). Isolation, in vitro characterization and predaceous activity of an indian isolate of the fungus, *arthrobotris oligospra* on the root-kone nematode, *meloidogyne incognita*. Nematol. medit., 33: 179-183.

Nagesh, M.; Hussaini, S.S.; Ramanujam, B. and Rangeswaran, R. (2007). Molecolar identification, characterization, variability and infectivity of indian isolate of the nematophagus fungus *pochonia chlamydosporia*. Nematol. medit, 35: 47-56.

Naik, M.K.; Hiremath, P.C. and Hegde, R. K. (1988). Physiological and nutritional studies on *Colletotrichum gloeosporioides*, A causal agent of anthracnose of beetlevine, Mysore J. Agric. Sci., 22:471-474.

Nordbring, H. B. (2004). Morphogenesis in the nematode-trapping fungus *Arthrobotrys oligospora*: an extensive plasticity of infection structures. Mycologist 18:125–133.

Pandey, V.S. (1973). Predatory activity of nematode trapping fungi against the larvae of *Trichostrongylus axei* and *Ostertagia ostertagi*: A possible method of biological control. J. Helminthol., 47(1): 35-48.

Papavizas,G.C.(1985).*Trichoderma* and *Gliocladium*: Biology, ecology, and potential for biological control. Ann. Rev. Phytopathol. 23: 23-54.

Rawsthorne, D. andd Hague, N.G.M. (1986) .The effect of *Heterodera avenae* on the root system of susceptible and resistant oat seedlings. Ann. Appl. Biol., 108: 89–98.

Sanial, P. K. (2000). Screening for Indian of predacious fungi for use in biological control against nematode parasites of ruminants. Vet. Res. Commun. 24: 55-62.

Saxena, G.; Dayal, R. and Mukerji, K.G. (1989). Nutritional studies on nematode-trapping fungi. Folia Microbiologica 34: 42–48.

Seyis, I., Aksoz, N. (2005). Investigation of Factors Affecting Xylanase Activity from *Trichoderma harzianum*. Brazilian archives of biology and technology. 48(n.2): 187-193.

Sharon, E.; Bareyal, M.; Chet, I.; Herreraestrella, A.; Kleifeld, O. and Spiegel, Y. (2001). Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Phytopathology 91:687–693.

Stirling, G.R. (1984). Biological control of Meloidogynejavanica with Bacillus penetrans. Phytopathology 74: 55-60.

Tunlid, T. and Sven, J. (1991). Proteases and Their Involvement in the Infection and Immobilization of Nematodes by the Nematophagous Fungus *Arthrobotrys oligospora*. Applied and environmental microbiology, p. 2868-2872.

Vogel, A.I. (1951) .Inorganic quantitative analysis, Longmans Green and Co., Toranto, 872p.

Zaki, M.J. and Maqbool, M. A. (1993). Effect of temperature and culture media on the growth of *Verticillium chlamydosporium*, an egg parasite of root-knot nematode and cyst nematodes. Pak J Phytopathol; 86: 102–105.