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Effects of Some Cultural Conditions on the Growth of Nematophagous Fungus *Pochonia chlamydosporia* (Fungi: Clavicipitaceae) Isolated from *Meloidogyne incognita* Eggs

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Abstract: The root-knot nematode, *Meloidogyne incognita* causes serious economic loss to tomato plantation in Turkey. Fungi associated with eggs of *M. incognita* in tomato plantation soil have not been studied yet but this knowledge could from the basis for future field studies on biological control of this nematode. In this study, effects of different pH levels, temperature and light intensity were tested on the growth of two isolates of nematophagous fungus *Pochonia chlamydosporia*: *Pochonia chlamydosporia* var. *chlamydosporia* and *Pochonia chlamydosporia* var. *catenulate*. These isolates were isolated from *M. incognita* eggs and analyzed based on their cultural conditions. The results of experiment indicated that the growth of *P. chlamydosporia* var. *chlamydosporia* was maximum in pH range of 4.00-7.00 and the best growth of *P. chlamydosporia* var. *catenulate* was in 6.00-7.00. The optimum temperature for growth ranged between 25 and 30°C for two isolates of *P. chlamydosporia*. Maximum growth of *P. chlamydosporia* var. *chlamydosporia* was in darkness and for *P. chlamydosporia* var. *catenulate* occurred in 12 h light and 12 h dark. Future studies are required to clarify the potential of these fungi as biological control agents of *M. incognita*.

Key words: *Pochonia chlamydosporia*, nematophagous fungus, cultural conditions, plantation, Turkey

INTRODUCTION

The nematodes are a very successful group of animals; they are important parasites of animals and plants and are of major medical and agricultural importance (Liu *et al*., 2009). Some soil-inhabiting fungi are nematophagous and have been used as biological control agents of harmful nematodes in agriculture (Stirling, 1991). 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 (Liu *et al*., 2009).

Major groups of nematophagous fungi are facultative parasites and have the capacity to colonize plant roots (Bordallo *et al*., 2002; Lopez-Llorca *et al*., 2002). Among the endoparasitic species, *Paecilomyces lilacinus* (Thom) Samson and *Pochonia chlamydosporia* (Goddard) Zaress and W. Gams are notable as biological control agents of *Meloidogyne* sp. (Kerry, 2001; Nagesh *et al*., 2005, 2007).

P. chlamydosporia is a facultative parasite of eggs of sedentary cyst nematodes (*Heterodera* sp.) and root-knot nematodes (*Meloidogyne* sp.) and has been associated with nematode suppressive soils (Kerry *et al*., 1993; Araujo *et al*., 2009; Arevalo *et al*., 2009). *P. chlamydosporia* is greatly distributed and has been successfully used in laboratory conditions for the parasite of eggs of *Meloidogyne* sp. (Hidaldo-Diaz *et al*., 2000a, b). This fungus can colonize the rhizosphere without causing lesions to the plants or affecting plant growth which represents an additional characteristic that facilitates its survival in soil in the absence of nematodes (De Leij and Kerry, 1991).

Production in great quantities of the fungus for biological control requires the best knowledge of the important factors: the most suitable pH level, temperature and light intensity. The objective of this research was to isolate parasitic fungi from *M. incognita* eggs and studied its cultural conditions.

MATERIALS AND METHODS

Preparations of samples: Ten randomized samples of 500 g of soil and infected roots were collected up to a depth of 10 cm from commercial tomato plantations (Kumluca-Turkey, 36°22.8'N; 30°16.8'E). The samples were placed in plastic bags and kept at 4°C until processed for isolation of nematode egg parasitic fungi. In the laboratory, roots of each sample were then washed,

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blotted dry, cut into 1 cm sections and mixed. About thirty egg-masses were picked up individually. Approximately 20 of them were mechanically disrupted before the eggs were suspended in 1 mL of 0.5% sterile agar solution, autoclaved at 121°C for 20 min and cooled at room temperature and 0.2 mL of the suspension plated onto water agar (12 g L^{-1}) containing 50 mg L^{-1} of each of the following antibiotics: streptomycin sulphate, chloramphenicol and chlortetracycline in two Petri dishes (9 cm diameter). They were incubated at 25°C for 24-48 h to enable fungi to grow out from colonized eggs (Kerry and Crump, 1977). Six colonized eggs were picked at random from each plate and subcultured on 12 g L^{-1} Corn Meal Agar (CMA) for 16 days at 25°C. The remaining 10 egg-masses were placed directly on a semi-selective medium for isolates of *P. chlamydosporia* (De Leij and Kerry, 1991) and incubated at 25°C for 10 days. The egg-masses colonized by fungi were preliminarily observed in open Petri dishes using an optical microscope (x100) to check for conidiophore branching patterns, arrangement of conidia and spore production. From these observations, species of egg parasitic fungi could be distinguished (Gams, 1988). Fungi field isolates were selected for detail studies on growth.

Fungal inoculation: For fungal inoculation, monosporic cultures were obtained for two isolates and stored at 4°C on 39 g L^{-1} Potato Dextrose Agar (PDA). Agar plugs (0.5 cm diameter) from the edge of a colony grown in PDA at 15°C for 14 days were placed on the centre of Petri dishes (9 cm diameter) containing 48 g L^{-1} Malt Extract Agar (MEA) and the plates incubated at 20°C in the dark. After 12 days, the dry mycelial weights were obtained using a Dikomsan HTSH precision scale (300-0.005 g). The isolates were analyzed and described using the keys and species descriptions proposed by Zare *et al.* (2001), Zare and Gams (2001).

Effect of pH: Effect of pH on the growth of those isolates was tested in the laboratory using liquid cultures containing different pH levels. Potato Dextrose Broth (PDB) medium was used to study the effect of pH of medium on the growth of two isolates of *P. chlamydosporia*. For this purpose, 25 mL 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 (1953). Flasks were sterilized at 121°C at 15 psi for 20 min. Each flask was inoculated with each isolate using 5 mm diameter mycelial disc in sterile conditions. Inoculated flasks were incubated at 25°C for 12 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 temperature: Temperature dependent linear growth rates for isolates compared. The fungi were subjected to different temperature conditions to study the best suited temperature level for the growth of the fungus. PDB medium was studied in one experiment to study the growth in liquid medium. A total of 25 mL of liquid medium was poured into a 150 mL conical flask under aseptic conditions and incubated with 5 mm diameter identical culture discs of each isolate. The experiment was done by five replicates. Inoculated conical flasks containing PDB medium were incubated at 10, 15, 20, 25 and 30°C. Dry mycelial weight was recorded in the liquid cultures 12 days after incubation.

Effect of light: The fungi cultures of two isolates on PDB were exposed to continuous light, dark and 12 h light and 12 h darkness in an environment chamber maintained at 25°C. Mycelial disk of 5 mm of each isolate was used to inoculate conical flask. Five replications were maintained for each treatment. Inoculated flasks were kept in environmental chamber and light intensity was adjusted to required level. The mycelia growth was recorded in each case 12 days after inoculation.

RESULTS AND DISCUSSION

Effect of pH: The mycelial growth was different among isolates and different pH levels (Table 1). The interaction between two isolates and pH levels was also significant ($p \leq 0.05$). In *P. chlamydosporia* var. *chlamydosporia* recorded maximum growth at pH 4-5 while pH 5-6 supported the maximum growth of *P. chlamydosporia* var. *catenulate*.

Effect of temperature: Temperature differences between two isolates were significant ($p \leq 0.05$). Two isolates of *P. chlamydosporia* responded differently to various temperature degrees as shown in the Table 2. Mean

Table 1: Growth of two isolates of *P. chlamydosporia* in different pH in PDB media. Mean mycelial weight (mg/25 mL)*

Isolate/pH	4	5	6	7	8
P. chlamydosporia var. chlamydosporia	161.5^{a}	153.1^{a}	148.3^{a}	141.1^{a}	93.4^{b}
P. chlamydosporia var. catenulata	166.5^{a}	174.3^{a}	179.1^{a}	169.3^{a}	119.2^{b}
Mean	164.0	163.7	163.7	155.2	106.3

*Values are means of five replicates. Values within a column or row followed by a same letter are significantly different at $p \leq 0.05$ according to Duncan's multiple range test

Table 2: Growth of two isolates of *P. chlamydosporia* in different temperatures in PDB media. Mean mycelial weight (mg/25 mL)*

Isolate/°C	10	15	20	25	30
P. chlamydosporia var. chlamydosporia	68.0^{b}	91.20^{b}	129.3^{a}	37.30^{c}	59.10^{c}
P. chlamydosporia var. catenulata	81.0^{b}	83.10^{b}	86.3^{b}	124.60^{a}	50.40^{c}
Mean	84.5	87.15	107.8	80.95	54.75

*Values are means of five replicates. Values are significantly different at p≤0.05 according to Duncan's multiple range tests

colony biomass of isolates on liquid medium was maximum at 15-25°C. Growth of *P. chlamydosporia* var. *chlamydosporia* was maximum at 15-20°C but *P. chlamydosporia* var. *catenulate* had better growth on 20-25°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 external factors which influence the growth of fungi, temperature plays an extremely important role.

Effect of light: Light has profound effect on the mycelial growth of nematophagous fungi. The exposure of the fungus to alternate cycles of 12 h light and 12 h darkness and darkness for 12 days resulted in the maximum mycelial growth of *P. chlamydosporia* var. *chlamydosporia*. The maximum growth of *P. chlamydosporia* var. *catenulate* was the best in continuous darkness (Table 3).

Growth at different cultural conditions was different for two isolates of *P. chlamydosporia*. The mycelial growth and sporulation of fungus are influenced by temperature. Kerry *et al.* (1993), Zaki and Maqbool (1993) and Nagesh *et al.* (2007) reported that the best temperature for growth of *P. chlamydosporia* was 25-35°C. The temperatures of 20 and 32°C affected equally the growth of *P. chlamydosporia* var. *chlamydosporia* whereas for *P. chlamydosporia* var. *catenulate* the temperature of 32°C caused lower growth than at 20°C (Arevalo *et al.*, 2009). These results are similar to this study for isolates. For other nematophagous fungi, Duponnois *et al.* (1995) and Nagesh *et al.* (2005) showed that optimum growth of *Arthrobotrys oligospora* occurred at 25-35°C. However, Morgan *et al.* (1997) recorded that peak growth rate was found to occur between 20 and 25°C for *A. chlamydosporia*.

Effect of pH on the growth of those isolates was also different. Nagesh *et al.* (2005) reported that the optimum pH ranged 6.0-7.5 for *A. oligospora* and optimum pH for *P. chlamydosporia* was 6.5-7.7. These results are different from this study pH values. For this study, results are pH 4.0-5.0 for *P. chlamydosporia* var. *chlamydosporia* and pH 5.0-6.0 for *P. chlamydosporia* var. *catenulate*. These differences could have related with culture medium. Kredics *et al.* (2003) demonstrated that isolate of Trichoderma can grown in pH 2.0-6.0 with an optimum

Table 3: Growth of two isolates of *P.chlamydosporia* in different light intensity in PDB media. Mean mycelial weight (mg/25 mL)*

Isolate/Light intensity	Light	Dark	12 h alternate light and dark
P. chlamydosporia var. chlamydosporia	127.00^{b}	158.40^{a}	168.30^{a}
P. chlamydosporia var. catenulata	121.70^{b}	153.10^{a}	129.20^{b}
Mean	124.35	155.75	148.75

*Values are means of five replicates. Values within a column or row followed by a same letter are significantly different at p≤0.05 according to Duncan's multiple range tests

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 and Aksoz (2005) observed that the activity of *Trichoderma harziamum* was maximum around pH 5.0. Kredics *et al.* (2003) demonstrated that Trichoderma strains are active under a wider range of pH. *A. oligospora* grew better on an acidic medium pH 5.6 (Duponnois *et al.*, 1995). For mycelial growth of *P. chlamydosporia* the optimal pH range was 5.0-6.0 with pH 6.0 giving the greatest biomass (Mo *et al.*, 2005).

CONCLUSION

One important aspect of nematophagous fungi is the possibility of using them for biological control of plant- and animal-parasitic nematodes. Plant parasitic nematodes, e.g., root knot (*Meloidogyne* sp.) and cyst nematodes (*Heterodera* sp.) are global pests in agriculture and horticulture, causing severe yield losses. Owing to the ban of many nematicides, e.g., methyl bromide because of health and environmental concerns, new alternatives for nematode control are therefore needed. Biological control may be such an alternative.

REFERENCES

Araujo, J.M., J.V. Araujo, F.R. Braga, R.O. Carvalho, A.R. Silva and A.K. Campos, 2009. Interaction and ovicidal activity of nematophagous fungus Pochonia chlamydosporia on *Taenia saginata* eggs. Exp. Parasitol., 121: 338-341.

Arevalo, J., L. Hidalgo-Dias, I. Martins, J.F. Souza, J.M.C. Castro, R.M.D.G. Carneiro and M.S. Tigano, 2009. Cultural and morphological characterization of *Pochonia chlamydosporia* and *Lecanicillium psalliotae* isolated from *Meloidogyne mayaguensis* eggs in Brazil. Trop. Plant Pathol., 34: 158-163.

Bordallo, J.J., L.V. Lopez Liorca, H.B. Jansson, J. Salines, L. Persmark and L. Asonsio, 2002. Colonization of plant roots by egg parasitic and nematode trapping fungi. New Phytol., 154: 491-499.

De Leij, F.A.A.M. and B.R. Kerry, 1991. The nematophagous fungus *Verticilium chlamydosporizkrn* as a potential biological control agent for *Meloidogyne arenaria*. Revue Nematol., 14: 157-164.

Duponnois, R., T. Mateille and M. Gueye, 1995. Biological characteristics and effects of two strains of arthrobotrys oligospora from senegal on meloidogyne species parasitizing tomato plants. Biocontrol. Sci. Technol., 5: 517-526.

Gams, W., 1988. A contribution to the knowledge of nematophagous species of *Verticillium*. Netherlands J. Plant Pathol., 94: 123-148.

Hidaldo-Diaz, L., J.M. Bourne and B.R. Kerry, 2000a. *Verticillium psalliotae* Treschews (Deuteromycotina: Hyphomycetes) as a parasite from eggs of *Meloidogyne* sp in Cuba: Characterization. Rev. Proteccion Veg., 15: 174-177.

Hidaldo-Diaz, L., J.M. Bourne, B.R. Kerry and M.G. Rodriguez, 2000b. Nematophagous *Verticillium* spp. in soils Infested with *Meloidogyne* sp. in Cuba: Isolated and screening. Int. J. Pest Manage., 46: 277-284.

Jackson, A.M., J.M. Whipps and J.M. Lynch, 1991. Biomass production of three *Trichoderma* isolates. J. Microbiol. Biotech., 7: 494-501.

Kerry, B.R. and D.H. Crump, 1977. Observation on fungal parasites of females and eggs of the cereal cyst nematode, *Heterodera avenae* and other cyst nematodes. Nematologica, 23: 193-201.

Kerry, B.R., 2001. Exploitation of the Nematophagous Fungus Verticillium Chlamydosporium Goddard for the Biological Control of Root-knot Nematodes (*Meloidogyne* spp.). In: Fungi as Biocontrol Agents: Progress, Problems and Potential, Butt, T.M., C. Jackson and N. Magan (Eds.). CABI Publishing, Wallingford, UK., pp: 155-168.

Kerry, B.R., I.A. Kirkwood, F.A.A.M. de Leij, J. Barba, M.B. Leidjens and P.C. Brookes, 1993. Growth and survival of *Verticillium chlamydosporium* Goddard, a parasite of nematodes in soil. Biocontrol Sci. Technol., 3:: 355-365.

Kredics, L., Z. Antal, L. Manczinger, A. Szekeres, F. Kevei and E. Nagy, 2003. Influence of environmental parameters on *Trichoderma* strains with biocontrol potential. Food Technol. Biotechnol., 41: 37-42.

Liu, X., M. Xiang and Y. Che, 2009. The living strategy of nematophagous fungi. Mycoscience, 50: 20-25.

Lopez-Llorca, L.V., J.J. Bordallo, J. Salinas, E. Monfort and M.L. Lopez-Serna, 2002. Use of light and scanning electron microscopy to examine colonisation of barley rhizosphere by the nematophagous fungus *Verticillium chlamydosporium*. Micron, 33: 61-67.

Mo, M.H., C.K. Xu and K.Q. Zhang, 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., J.M. Behnke, J.A. Lucas and J.F. Peberdy, 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*. Mycopathologia, 115: 303-310.

Nagesh, M., S.S. Hussaini, B. Ramanujam and R. Rangeswaran, 2007. Molecular identification, characterization, variability and infectivity of Indian isolate of the nematophagous fungus *Pochonia chlamydosporia*. Nematol. Medit., 35: 47-56.

Nagesh, M., S.S. Hussaini, B.S. Chidanandaswamy and S.R. Biswas, 2005. Isolation *in vitro* characterization and predaceous activity of an Indian isolate of the fungus *Arthrobotrys oligospora* on the root-knot nematode, *Meloidogyne incognita*. Nematol. Medit., 33: 179-183.

Naik, M.K., P.C. Hiremath and R.G. Hedge, 1988. Physiological and nutritional studies on *Colletotrichum gloeosporioides*, A casual agent of anthracnose of beetle vine. Mysore J. Agric. Sci., 22: 471-474.

Nordbring-Hertz, B., 2004. Morphogenesis in the nematode-trapping fungus *Arthrobotrys oligospora*-An extensive plasticity of infection structures. Mycologist, 18: 125-133.

Seyis, I. and N. Aksoz, 2005. Investigation of factors affecting xylanase activity from *Trichoderma harzianum*. Braz. Arch. Biol. Technol., 48: 187-193.

Stirling, G.R., 1991. Biological Control of Plant Parasitic Nematodes: Progress, Problems and Prospects. CAB International, Wallingford, UK., Pages: 282.

Vogel, A.L., 1953. A Text-Book Of Quantitative Inorganic Analysis: Theory and Practice. Longans, Green and Co., London.

Zaki, M.J. and M.A. Maqbool, 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.

Zare, R. and W. Gams, 2001. A revision of *Verticillium* section prostrata. IV. The genera *Lecanicillium* and *Simplicium* gen. nov. Nova Hedwigia, 73: 1-50.

Zare, R., W. Gams and H.C. Evans, 2001. A revision of *Verticillium* section prostrate. V. The genus *Pochonia*, with notes on *Rotiferophthora*. Nova Hedwigia, 73: 51-86.