Appl. Ent. Phytopath.
Vol. 77, No. 2, March 2010

# *In vitro* assessment of pathogenicity and culture filtrates of fungi against *Heterodera schachtii*

**E. AYATOLLAHY[1] and S. FATEMY[2]***

1- Department of Plant Protection, Abourayhan Campus, University of Tehran

2- Iranian Research Institute of Plant Protection, Tehran

(Received: July 2008; Accepted: May 2009)

**Abstract**

The pathogenicity of four isolates of three fungal species against females and cysts of *Heterodera schachtii* on water agar and the effects of fungal filtrates on motility of second-stage juveniles were examined. Rates of egg colonization by the fungi were measured after 3 weeks at 20 °C on 0.8% water agar. Fungi penetrated both females and cysts of which 60, 36, 21 and 17% of the eggs were infected by the two isolates of *Paecilomyces lilacinus* (PL 8.1 & PL 25.4), *Cylindrocarpon destructans* var. *crassum* and *Chaetomium murorum*, respectively. Filtrates of the fungi reduced mobility of the nematode $J_2$ after 24 and 48 h incubation periods. Isolate 8.1 of *P. lilacinus* paralyzed 100% of the $J_2$ after both 24 and 48 h incubation periods. After 48 h of incubation the extracts of isolate 25.4 of *P. lilacinus*, *C. destructans* var. *crassum* and *C. murorum* paralyzed 25, 22 and 9% of the $J_2$, respectively.

**Key words:** *Chaetomium murorum*, *Cylindrocarpon destructans* var. *crassum*, *Paecilomyces lilacinus*, fungal filtrate, juvenile mobility, sugar beet cyst nematode.

**چکیده**

بیماری‌زایی ۴ جدایه متعلق به سه گونه قارچ جدا شده از *Heterodera schachtii* علیه سیست و ماده‌های شیری بر روی آب آگار و اثرات کشندگی پالیده‌های (عصاره) آن‌ها روی لاروهای سن دو نماتد مورد ارزیابی قرار گرفت. این جدایه‌ها شامل *Paecilomyces lilacinus*

* **Corresponding author:** sfatemy@yahoo.com

(PL 8.1 and PL 25.4)، *Cylindrocarpon destructans* var. *crassum* (CY) و *Chaetomium murorum* (CH) پس از سه هفته روی محیط آب آگار ۸/ ٪ در دمای ۲۰°C با نفوذ به ماده‌ها و سیست‌ها به ترتیب ۶۰، ۳۶، ۲۱ و ۱۷٪ از تخم‌های درون آن‌ها را بیمار نمودند ($P \leq 0.01$).

پالیده کشت جدایه‌ها سبب کاهش تحرک نماتدها بعد از ۲۴ و ۴۸ ساعت گردید. پالیده کشت PL 8.1 در محیط مایع مالت بعد از ۲۴ و ۴۸ ساعت ۱۰۰٪ لاروهای نماتد را از بین برد و جدایه‌های PL 25.4، CY و CH به ترتیب سبب مرگ و میر ۲۵، ۲۲ و ۹٪ لاروها پس از گذشت ۴۸ ساعت گردیدند*.

**واژه‌های کلیدی:** *Chaetomium murorum*، *Cylindrocarpon destructans* var. *crassum*، پالیده قارچ، مرگ و میر لارو، *Paecilomyces lilacinus*، نماتد سیست چغندرقند.

**Introduction**

Fungi can directly parasitize nematodes (Siddiqui & Mahmood, 1996; Holland *et al*., 1999; Olivares- Bernabeu & Lopez-LIorca, 2002; Chen & Chen, 2003; Fatemy *et al*., 2005) or secrete nematicidal metabolites and enzymes that affect nematode viability (Cayrol *et al*., 1989; Nitao *et al*., 1999; Chen *et al*., 2000). These active compounds have potential for application as novel nematicides (Meyer *et al*., 2004).

Toxic effects of fungal culture filtrates on vermiform nematodes and eggs have been reported in a number of studies from several fungi such as species of *Paecilomyces*, *Pochonia* (*Verticillium*), *Fusarium*, *Aspergillus*, *Trichoderma*, *Myrothecium* and *Penicillium*. Many more soil fungi that are antagonistic to nematodes through the release of toxins, antibiotics or enzymes remain to be discovered (Chen & Dickson, 2004).

Culture filtrates of *Paecilomyces lilacinus* Samson were toxic to nematodes (Cayrol *et al*., 1989; Chen *et al*., 2000; Khan & Goswami, 2000). The leucinostatins in *P. lilacinus* are indicators of nematicidal activity, whereas chitinase activity might be related to parasitism (Park *et al*., 2004) and can cause undifferentiated eggs of *Meloidogyne hapla* Chitwood,

* **نشانی نگارندگان:** مهندس ابراهیم آیت‌اللهی، گروه بیماری‌شناسی گیاهی، دانشکده ابوریحان، دانشگاه تهران، ایران؛ دکتر صدیقه فاطمی، بخش تحقیقات نماتدشناسی، مؤسسه تحقیقات گیاهپزشکی کشور، صندوق پستی ۱۴۵۴، تهران ۱۹۳۹۵، ایران.

1949 to become deformed and vacuolated (Fitters *et al*., 1992). Also, a peptide antibiotic, P-168, has been isolated from cultures of *P. lilacinus* (Isogai *et al*., 1980). A species of *Chaetomium* produced a low molecular weight compound as flavipin that *in vitro* inhibited egg hatch and juvenile mobility of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 and *Heterodera glycines* Ichinohe, 1952 and was responsible for most of the nematode-antagonistic activity (Nitao *et al*., 2002). An antibiotic from *Cylindrocarpon* showed good nematicidal activity and had a low toxicity towards vertebrates (Coosemans, 1991).

In Iran, *P. lilacinus*, *Cylindrocarpon* spp. and *Chaetomium murorum* have been isolated from eggs of sugar beet cyst nematode, *Heterodera schachtii* Schmidt, 1871 (Fatemy *et al*., 1999).

In this study, the bioactivity of two isolates of *P. lilacinus* (2 isolates) and *Cylindrocarpon destructans* var. *crassum* (Wollenweber) C. Booth, and *Chaetomium murorum* were investigated against females and cysts of *H. schachtii* on agar, further more, toxic activity of fungal filtrates were tested against second-stage juveniles *in vitro*.

**Materials and Methods**

Fungal isolates: Fungal strains which had been isolated from eggs and juveniles of *H. schachtii* in Khorasan Province (Fatemy *et al*. 1999), were maintained on potato dextrose agar (PDA) at 5 °C until required.

Nematode inoculum: To prepare *H. schachtii* inoculum, infected soil and roots from a three months culture of sugar beet (*Beta vulgaris* L.) cultivar IC1 which had been inoculated with a cyst of nematode, were removed from the pots in the greenhouse, the cysts and females were washed from wet soil, passed through 850 and 250 µm sieves; collected into a beaker and separated from soil particles and debris by the centrifugal floatation technique (Jenkins, 1964; Kim & Riggs, 1995).

For obtaining live second-stage juveniles, cysts were surface-sterilized with 0.1% NaOCl (commercial bleach) for 30 minutes, rinsed with plenty of sterile distilled water, placed on a filter paper on a screen in a dish containing 150 ml of a 4 mM $ZnCl_2$ artificial hatching solution (Clarke & Shepherd, 1964) and incubated at room temperature (23-28°C). Newly hatched $J_2$ were collected daily and kept at 5°C until used.

Temperature test: To evaluate the optimum temperature at which the greatest growth of the fungi occur, discs of 5-mm-diameter were cut from the edge of each actively growing

fungal colony on PDA and one plug was transferred to the centre of each of 9-cm-diameter Petri dish containing 15 ml PDA. Three replicates for each fungus were arranged randomly in incubators at 5, 10, 15, 20, 25, 30 or 35°C. After 10 days, with the aid of a stereomicroscope, colony growth was estimated by measuring two diameters crossing the centre of the dish at right angles.

Pathogenicity test: To surface-sterilize, cysts and females were first dipped in 0.1% NaOCl (commercial bleach) for half an hour, rinsed with distilled water, placed in a solution containing 100 ppm of tetracycline, streptomycin sulphate and choloramphenicol for 30 minutes; rinsed five times with sterile distilled water and placed on potato dextrose agar for 48 hours; only uninfected females and cysts were used for experiments. The centre of 5-cm-diameter Petri dishes, containing 11 ml of 0.8% water agar and 100 ppm each of streptomycin sulphate, choloramphenicol and tetracycline, were inoculated with a 5-mm-diameter disc cut from the edge of actively growing fungal colonies on PDA. Uninfected PDA plugs were added to control dishes. Three to four females or cysts were placed close to the plugs and all dishes were arranged randomly in an incubator at 20°C in the dark. After three weeks, each female or cyst was placed in a drop of lactoglycerol solution (lactic acid 85%: glycerol 99.5%: distilled water = 2:2:1) on a slide, crushed by a cover slip and the contents were spread for examination of the eggs; infected and dead eggs became clearer within a minute in the solution, whereas uninfected eggs remained unchanged and darker in appearance (Kim & Riggs, 1994). The proportion of infected eggs, unhealthy looking, healthy immature and mature eggs were determined using a light microscope at 200× and 400× magnification and the number of emerged juveniles in each dish were counted using a stereomicroscope at 50× magnification (Fatemy *et al*., 2005)

Culture filtrate test: Two discs of 1-cm-diameter, taken from the margin of a fungal colony actively growing on corn meal agar, were added to flasks of 500 ml, each containing 250 ml of 1.5% malt extract broth (Merck). Along with uninoculated flasks, they were sealed with cotton and aluminum foil and placed on a reciprocating shaker at low speed at room temperature (23-27°C) in the dark. After two weeks, the content of each flask was filtered through a filter paper (Whatman No. 1) and a 0.22-µm Millipore filter to remove bacteria and fungal spores.

Freshly hatched second-stage juveniles of the nematode were collected on a 20 micrometer filter, were surface-sterilized by dipping in 1% streptomycin sulphate solution for an hour and rinsed in sterile distilled water several times. An aliquot of 0.1 ml suspension,

containing ca. 50 juveniles, and 1 ml of undiluted culture filtrate of each fungus were added to each well of tissue culture plates. Distilled water and the medium without fungus were included as controls. Three replicates were allocated for each treatment and plates were maintained at 25°C. Viability of the $J_2$s was examined after 24 and 48 hours by using the technique described by Chen & Dickson (2000); with an inverted microscope. Generally, two drops of 1N NaOH were added to each well, nematodes that changed their body shape from straight to curled or walking-stick shape within 3 minutes of application were considered alive, whereas those that failed to respond to NaOH application were regarded as paralyzed or dead. Nematodes were then transferred to water, to further check their recovery.

Statistical analysis: The experiments were repeated and data which are means of two tests were checked for homogeneity of variance and pooled only when variance homogeneity could be assumed. The percentage of diseased eggs and paralyzed J2 were transformed by arc sin ( $\sqrt{x}$ ) before being subjected to ANOVA. Duncan's multiple- Range test at $P \leq 0.01$ was used to compare the means (Little & Hills 1978).

**Result and Discussion**

Temperature test: The maximum growth of the fungi occurred at 30°C for both isolates of *P. lilacinus* and at 20°C for *C. destructans* var. *crassum* and *Chaetomium murorum.* ($P \leq 0.01$) (Fig. 1). At 5°C, only *C. murorum* had a measurable growth. The growth of this fungus reached 32 mm at 20 °C and decreased at higher temperatures. Of *P. lilacinus,* isolate 25.4 did not start growing below 15°C, whereas colony of strain 8.1 measured 5-mm at 10°C. *Cylindrocarpon destructans* grew 3-mm at 10°C, reached a maximum growth of 36-mm at 20 and decreased at higher temperatures. None of the fungi grew at 35°C.

Pathogenicity test: The mycelium of *P. lilacinus* infected eggs of *H. schachtii* (Fig. 2), and nearly 61% and 36% of the eggs within females and cysts were killed by isolates 8.1 and 25.4, respectively ($P \leq 0.01$) (Table 1). Eggs colonized by *C. destructans* var. *crassum* were yellow to light brown in color (Fig. 2) and 21% of the eggs in females and 3% of those in cysts were colonized by this fungus. The mycelium of *C. murorum* was observed in 13 and 17% of the eggs in females and cysts, respectively ($P \leq 0.01$) (Table 1).

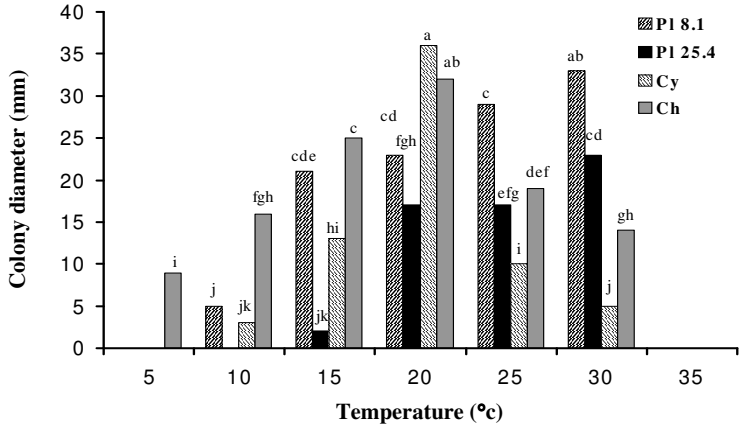


**Fig. 1-** Effect of different temperatures on fungal colony growth on PDA after 10 days. Data are means of three replicates. Figures with the same letters are not significantly different by Duncan's test at 1% level. PL 8.1 and PL 25.4: *Paecilomyces lilacinus*, CY: *Cylindrocarpon destructans* var. *crassum*, CH: *Chaetomium murorum*

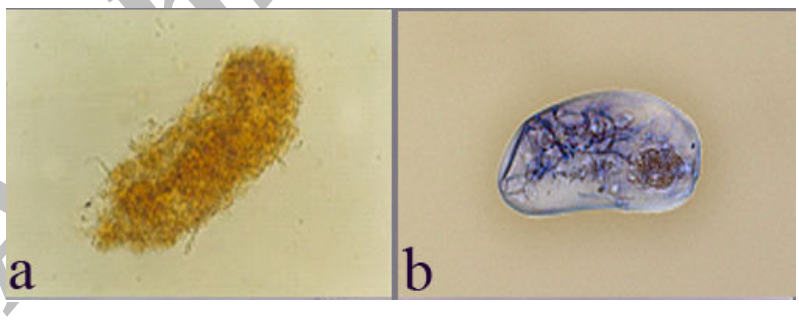


**Fig. 2-** Immature eggs of *Heterodera schachtii* infected by fungi; a: *C. destructans* var. *crassum* (400×); b: *P. lilacinus* (400 ×)

**Table 1-** Pathogenicity of fungi on eggs within cysts and females of *H. schachtii* after 21 days at 20 °C on water agar

| Fungus | Colonized eggs (%) | |
|---|---|---|
| | Females | Cysts |
| *P. lilacinus* (8.1) | 60.7 a | 61.3 a |
| *P. lilacinus* (25.4) | 36.2 ab | 35.6 b |
| *C. destructans* var. *crassum* | 20.8 bc | 3.3 d |
| *Chaetomium murorum* | 12.9 bc | 16.7 c |
| Control | 0.0 c | 0.0 |

Data are means of three replicates. Figures in each column followed by the same letter are not significantly different at 1% level by Duncan's test. The data were transformed by arc sin ( $\sqrt{x}$ ).

**Table 2-** Percentage of immobile second-stage juveniles of *H. schachtii* incubated in original culture filtrates of fungi after 24 and 48 h. Fungi were cultured in malt extract broth for two weeks at 23-27°C in darkness on a shaker

| Fungi | Incubation period (hours) | |
|---|---|---|
| | 24 | 48 |
| *P. lilacinus* (8.1) | 100 a | 100 a |
| *P. lilacinus* (25.4) | 13.8 bc | 24.7 b |
| *C. destructans* var. *crassum* | 18.7 b | 21.5 b |
| *Chaetomium murorum* | 8.9 c | 9.2 c |
| Uninoculated culture filtrate | 3.0 d | 6.2 c |
| Distilled water | 2.1 d | 4.4 c |

Data are means of three replicates. Figures in each column followed by the same letters are not significantly different at 1% level by Duncan's test. The data were transformed by arc sin ( $\sqrt{x}$ ).

Culture filtrate test: Culture filtrates of different fungi had significantly (P≤0.01) different effects on the mobility of the nematode juveniles. After 24 h, 100% and 14% of juveniles became motionless in original solutions of *P. lilacinus* isolate 8.1 and 25.4, respectively, and the number of inactive juveniles increased to 25% after 48 h in filtrate of isolate 25.4 (P≤ 0.01) (Table 2). Filtrates of *C. destructans* var. *crassum* decreased motility of juveniles between 19 to 22% after 24 and 48 h, respectively (P≤ 0.01) (Table 2). The proportion of immobile juveniles in the filtrate of the *C. murorum* was 9% which did not change over time.

In our study, all fungi infected eggs of *H. schachtii* to some degree, produced substances toxic to juveniles. *Paecilomyces lilacinus* (isolate 8.1) killed nearly 100% of the juveniles after 24 h. Earlier studies demonstrated that culture filtrate of this fungus on malt extract broth paralyzed 100% of second-stage juveniles of *H. glycines* after 24 h (Chen *et al*., 2000). On the other hand, isolate 25.4 of this fungus immobilized less J2 (25%) under the same conditions in our study. These differences have also been encountered by other researchers. Culture extracts produced by strains of *Pochonia chlamydosporia* (Goddard) Zare & W. Gams were toxic to nematodes in some experiments (Caroppo *et al*., 1990), but not significantly toxic in other experiments (Chen *et al*., 2000; Ayatollahy *et al*., 2008). The fungal strains, nematode species, culture media and experimental conditions are possible explanations for the observed differences (Hallmann & Sikora, 1996; Hojat-Jalali, 1998; Chen *et al*., 2000).

While *P. lilacinus* was considered a weak colonizer of eggs of *H. glycines* (Chen *et al*., 1996) or *H. schachtii* (Hojat-Jalali, 1998), in this study, isolate 8.1 of *P. lilacinus* parasitized more than 60 % of eggs in females and cysts and was capable of infecting juveniles in eggs within cysts.

*Cylindrocarpon destructans* var. *crassum* was moderately parasitic in our study and on eggs of *H. glycines* (Chen & Chen, 2003) whereas it was particularly effective in decreasing potato cyst nematode populations (Crump & Flynn, 1995). The results of present experiments with *C. murorum* were similar to those of other researchers (Rodriguez-Kabana & Morgan-Jones, 1988) in that the fungus was a weak colonizer of the eggs and its filtrates had some toxic effects on *H. schachtii* second stage juveniles. An antibiotic isolated from *Cylindrocarpon olidum* Wollenweber showed large nematicidal activity (Coosemans, 1991) and *Chaetomium* isolates had toxic activity against *M. incognita* and *H. glycines* (Nitao *et al*., 2002). Fungi exhibit a range of specificities and mode of action in their antagonistic

activity towards nematodes; for example, some strains of *P. lilacinus* have antagonistic activity against fungi and bacteria (Brian & Hemming, 1947) and toxins may enable the fungus to compete with soil micro organisms.

Results of our experiments agree with previous findings that active nematicidal compounds are produced by *Fusarium equiseti* (Corda) Sacc. (Nitao *et al*., 1999), *Pochonia suchlasporia* (W. Gams & Dackman) Zare & W. Gams (Lopez-Llorca and Boag, 1993), *Penicillium* sp. and *Aspergillus niger* (Molina & Davide, 1986). In searching for an efficient and environmentally friendly method of nematode management, interest has been increased in this line of research. Therefore, more research is necessary to develop a rapid and efficient bioassay procedure for screening large number of fungal metabolites against nematodes.

**Acknowledgements**

This study is part of a research project of the Plant Protection Research Institute, Ministry of Agriculture, Iran, which has been submitted as an MSc thesis by the first author. The authors thank Dr. S. Y. Chen, Southern Research and Outreach center, University of Minnesota and Prof. J. Coosemans, Fytopathologie Plantenbescherming, K.U., Leuven, Belgium, for their useful comments.

**References**

AYATOLLAHY, E., S. FATEMY and H. R. ETEBARIAN, 2008. Potential for biological control of *Heterodera schachtii* by *Pochonia chlamydosporia* var. *chlamydosporia* on sugar beet. Biocont. Sci. Technol. 18: 157-167.

BRIAN, P. W. and H. G. HEMMING, 1947. Production of anti fungal and antibacterial substances by fungi, preliminary examination of 166 strains of Fungi Imperfecti. J. General Microbiology 1: 158-167.

CAROPPO, S., B. PERITO and O. PELAGATTI, 1990. *In vitro* evaluation of nematicide activity by several fungi against *Meloidogyne incognita* eggs. Redia 73: 451-462.

CAYROL, J. C., C. DJIAN and L. PIJAROWSKI, 1989. Study of the nematicidal properties of the culture filtrate of the nematophagous fungus *Paecilomyces lilacinus*. Rev. Nématol. 12: 331-336.

CHEN, S. Y. and F. J. CHEN, 2003. Fungal parasitism of *Heterodera glycines* eggs as influenced by egg age and pre-colonization of cysts by other fungi. J. Nematol. 35: 271-

277.

CHEN, S. Y. and D. W. DICKSON, 2000. A technique for determining live second-stage juveniles of *Heterodera glycines*. J. Nematol. 32: 117-121.

CHEN, S. Y. and D. W. DICKSON, 2004. Biological control of nematodes by fungal antagonists. Pp. 979-1039. *In*: Nematology, Advances and Perspectives-Vol. 2. (Chen Z. X, Chen S. Y. and Dickson D. W., eds). CABI Publishing, Wallingford, UK.

CHEN, S. Y., D. W. DICKSON and D. J. MITCHELL, 1996. Pathogenicity of fungi to eggs of *Heterodera glycines*. J. Nematol. 28 : 148-158

CHEN, S. Y., D. W. DICKSON and D. J. MITCHELL, 2000. Viability of *Heterodera glycines* exposed to fungal filtrates. J. Nematol. 32: 190-197.

CLARKE, A. J. and A. M. SHEPHERD, 1964. Synthetic hatching agents for *Heterodera schachtii* Schmidt and their mode of action. Nematologica 10: 431-453.

COOSEMANS, J. 1991. Antagonistic activity of a *Cylindrocarpon olidum* (Wollen.) isolate against nematodes. Meded: Fac. Landbouww. Rijksuniv. Gent 56: 223-228.

CRUMP, D. H. and C. A. FLYNN, 1995. Isolation and screening of fungi for the biological control of potato cyst nematodes. Nematologica 41: 628-638.

FATEMY, S., A. AHMADIAN, R. PARVIZI, A. AHMADI, M. PAKNIAT, S. BAROOTI, M. ASKARI and J. ERSHAD, 1999. Fungal parasites of cysts of *Heterodera schachtii* in Iran. Pak. J. Nematol. 17: 61-66.

FATEMY, S., F. SAEIDI-NAEINI and A. ALIZADEH, 2005. *In vitro* screening of fungi for parasitism against sugar beet cyst nematode *Heterodera schachtii.* Nematol. Medit. 33: 185-190.

FITTERS, P. F. L., E. BELDER and E. DEN-BELDER, 1992. Effects of fungal products on nematodes. Gewasbescherming 23: 16 (Abstr.).

HALLMANN, J. and R. A. SIKORA, 1996. Toxicity of fungal endophyte secondary metabolites to plant parasitic nematodes and soil-born plant pathogenic fungi. Eur. J. Pl. Path. 102: 155-162.

HOJAT-JALALI, A. A. 1998. Study of antagonistic fungi of beet cyst nematode, *Heterodera schachtii* from Iran. Ph.D thesis, KU Leuven. 226 pp.

HOLLAND, R. J., K. L. WILLIAMS and A. KHAN, 1999. Infection of *Meloidogyne javanica* by *Paecilomyces lilacinus.* Nematology 1: 131-139.

ISOGAI, A., A. SUZUKI, S. HIGASHIKAWA, S. KUYAMA and S. TAMURA, 1980. Constituents of a peptidal antibiotic P168 produced by *Paecilomyces lilacinus* (Thom)

Samson. Agric. Biol. Chem. 44: 3029-3031.

JENKINS, W. R. 1964. A rapid centrifugal floatation technique for separating nematodes from soil. Pl. Dis. Rep. 48: 692.

KHAN, M. R. and B. K. GOSWAMI, 2000. Effect of culture filtrates of *Paecilomyces lilacinus* isolates on hatching of *Meloidogyne incognita* eggs. Ann. Pl. Prot. Sci. 8: 62-65.

KIM, D. G. and R. D. RIGGS, 1994. Techniques for isolation and evaluation of fungal parasites of *Heterodera glycines*. J. Nematol. 26 (supplement): 592-595.

KIM, D. G. and R. D. RIGGS, 1995. Portable cyst extractor: detecting cyst nematodes in the field. J. Nematol. 27: 125-126.

LITTLE, T. M. and F. J. HILLS, 1978. Agricultural Experimentation Design and Analysis. John Willey & Sons, Inc. N Y, USA. 349 pp.

LOPEZ-LLORCA, L. V. and B. BOAG, 1993. Biological properties of a red pigment produced by the nematophagous fungus *Verticillium suchlasporium*. Nematol. Medit. 21: 143-149.

MEYER, S. L. F., R. N. HUETTEL, X. Z. LIU, R. A. HUMBER, J. JUBA and J. K. NITAO, 2004. Activity of fungal culture filtrates against soybean cyst nematode and root-knot nematode egg hatch and juvenile motility. Nematology 6: 23-32.

MOLINA, G. C. and R. G. DAVIDE, 1986. Evaluation of microbial extracts for nematicidal activity against plant parasitic nematodes *Meloidogyne incognita* and *Radopholus similis*. The Philippine Agriculturist . 69: 173-186.

NITAO, J. K., S. L. F. MEYER and D. J. CHITWOOD, 1999. *In vitro* assays of *Meloidogyne incognita* and *Heterodera glycines* for detection of nematode-antagonistic fungal compounds. J. Nematol. 31: 172-183.

NITAO, J. K, S. L. F. MEYER, J. E. OLIVER, W. F. SCHMIDT and D. J. CHITWOOD, 2002. Isolation of flavipin, a fungus compound antagonistic to plant parasitic nematodes. Nematology 4: 55-63.

OLIVARES-BERNABEU, C. and L. V. LOPEZ-LLORCA, 2002. Fungal egg-parasites of plant parasitic nematodes from Spanish soils. Rev. lberoam Micol. 19: 104-110.

PARK, J. O., J. R. HARGREAVES, E. J. MCCONVILLE, G. R. STIRLING, E. L. GHISALBERTI and K. SIVASITHAMPARAM, 2004. Production of leucinostatins and nematicidal activity of Australian isolates of *Paecilomyces lilacinus* (Thom) Samson. Letters in Applied Microbiology 38: 271-276.

RODRIGUEZ-KABANA, R. and G. MORGAN-JONES, 1988. Potential for nematode control by mycofloras endemic in the tropics. J. Nematol. 20: 191-203.

SIDDIQUI, Z. A. and I. MAHMOOD, 1996. Biological control of plant parasitic nematodes by fungi: A review. Bioresearch Technol. 58: 229-239.

---

**Address of the authors:** Eng. E. AYATOLLAHY, Department of Plant Protection, Abourayhan Campus, University of Tehran, Iran; Dr. S. FATEMY, Department of Nematology, Iranian Research Institute of Plant Protection, P. O. Box 1454, Tehran 19395, Iran.