The African Journal of Plant Science and Biotechnology ©2011 Global Science Books

Isolation of Non-pathogenic *Fusarium* spp. Strains for Biological Control of *Meloidogyne incognita* on Tomato

Hajer Regaieg[1*] • Najet Horrigue-Raouani[1] • Mejda Daami-Remadi[2]

[1] Institut Supérieur Agronomique de Chott-Mariem, 4042 Chott-Mariem, Université de Sousse, Tunisia
[2] Centre Régional des Recherches en Horticulture et Agriculture Biologique, Chott-Mariem 4042, Université de Sousse, Tunisia
Corresponding author: * **regaieghajer@yahoo.fr**

ABSTRACT

This study was conducted to investigate the effects of three isolates of *Fusarium* spp. (HR4, HR23 and HR57) on *Meloidogyne incognita* (Kofoid and White), and their pathogenicity on tomato (*Lycopersicon esculentum* Mill.) plants. *Fusarium* isolates were obtained from egg masses extracted from tomato plants grown in plastic greenhouses in Békalta (centre of Tunisia) and identified by sequencing of the internal transcribed spacer fragments (ITS). Healthy *M. incognita* eggs were placed on fungal colonies already formed on water agar. The highest infection rate was observed 7 days after inoculation and reached 70%. *F. oxysporum* had the ability to penetrate through the eggshell. The effects of *Fusarium* spp. on *M. incognita* in tomato plants were tested in pot experiments in growth chambers. A positive effect was observed on plant height fresh shoot and root weight. The number of galls and egg masses in the root system decreased within 45 days after inoculation of *Fusarium* spp. strains.

Keywords: biological control, *Fusarium* spp., *M. incognita*, tomato
Abbreviations: DNA, deoxyribonucleic acid; **PCR**, polymerase chain reaction; **PDA**, potato dextrose agar

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important commercial and widely grown vegetable crop in many area of the Mediterranean region, which is often severely attacked by several pests. Root knot nematodes (*Meloidogyne* spp.) are one of the major pathogens of tomato worldwide (Sikora and Fernández 2005). Control of plant parasitic nematodes is currently limited to application of nematicides, which are costly and detrimental to the environment and human health (Wachira *et al*. 2009). Therefore, alternative control measures for the management of root knot nematodes are needed.

Biological control using endophytic microorganisms has been demonstrated to be highly effective against sedentary and migratory endoparasites that complete their life-cycle inside the host (Sikora 1992; Sikora *et al.* 2007). Some endophytic *Fusarium* strains are known to be pathogens on certain crops and induce either root rot or tracheomycosis (Alabouvette *et al*. 1998); however, the majority of strains are non-pathogenic saprophytes that inhabit soil (Alabouvette *et al.* 1998; Fravel *et al.* 2003). High interest is being given to the study of non-pathogenic strains of *Fusarium* in the biocontrol of soil-borne pathogens (Olivain *et al*. 2006; Minerdi *et al*. 2008; Kaur *et al*. 2010) as well as root-knot nematode (Vu *et al*. 2004; Yan *et al*. 2011). Only a few species of *Fusarium* have been tested in the laboratory and the greenhouse for their potential as biocontrol agents against nematodes. A number of *Fusarium* species have been isolated from females, cysts, egg masses and eggs of nematodes (Dos Santos *et al.* 1992; Sun *et al*. 2006; Ashoub *et al*. 2009). Many non-pathogenic *Fusarium* strains have been isolated from asymptomatic roots in nematode-infested fields where they grow without causing any visible damage to the plant; such strains were isolated from many crops including rice, cucumber, tomato or banana (Hallman and Sikora 1994; Huang *et al.* 2009; Yan *et al.* 2011). In these biocontrol systems, the fungi suppressed nematode activity both at invasion by decreasing the nematode penetration and during development by reducing the gall index and the number of egg masses on roots (Hallman and Sikora 1994).

The objective of the current investigation was to evaluate the potential of three *Fusarium* isolates obtained from egg masses removed from tomato roots showing gall formation as biological control agents against *M. incognita*.

MATERIALS AND METHODS

Isolation and identification of *Fusarium* isolates by ITS gene sequencing

Prior to the molecular characterization, all isolates were identified macroscopically and microscopically based on their morphological traits on potato dextrose agar medium (PDA) using the criteria recommended by Domsch *et al.* (1980).

1. Fungal isolates

Isolates of *Fusarium* spp. (HR4, HR23 and HR57) were obtained from tomato roots heavily infested with *Meloidogyne* spp. collected from several greenhouses in centre of Tunisia (Békalta). Egg masses were picked up from the root surface and surface disinfected with 1% sodium hypochlorite (NaOCl) for 30 s. They were collected on a 30-µm aperture sieve and washed with sterile distilled water to remove residual NaOCl. About 5 egg masses were smeared on each Petri dish containing PDA and incubated at 23°C for one week. Eggs were daily examined for colonization of fungi using an inverted microscope. Hyphae growing from egg masses were transferred to PDA plates for purification and identification.

2. *DNA isolation*

For DNA extractions, all *Fusarium* spp. isolates were grown on PDA for 7 days at 25°C. Mycelium was then scraped directly from

Received: 10 December, 2010. Accepted: 24 May, 2011.

Original Research Paper

agar plates and used for DNA isolation. Cultures were transferred to a 1.5 ml Eppendorf tube homogenized with a pestle in 0.7 ml 2X CTAB buffer. Eppendorf tubes were incubated at 65°C for 30 min, and then 0.7 ml of chloroform was added and mixed briefly. After centrifugation at 15000 rpm for 30 min, the supernatant was transferred into a new tube mixed with 0.6 ml isopropanol and chilled to 20°C, followed by another centrifugation step for 5 min at 15000 rpm. The supernatant was discarded and the remaining pellet was washed twice with 1 ml of 70% ethanol, followed by drying under vacuum and thereafter dissolved in 1 ml TE (10 mM Tris, 1 mM EDTA, pH 7.5) buffer. The DNA was visualized on a 1% agarose gel (wt/v) (Boehringer Mannheim) stained with SYBR-safe (Invitrogen, USA) and viewed under ultra-violet light. DNA concentrations were estimated by comparing the intensity of SYBR-safe fluorescence of the DNA sample to a known concentration of lambda DNA marker (marker III, Roche Diagnostics, Mannheim, Germany).

3. PCR amplification of ribosomal DNA regions

Isolated DNA (50 ng) was used as template for the PCR reaction. The ITS1 and ITS2 and the inverting 5.8S coding rDNA were amplified by PCR using the primers ITS1 (5′-TCCGTAGGTGAA CCTGCGG-3′) and ITS4 (5′-GGAAGTAAAAGTCGTAACAAG G-3′) as described by White *et al.* (1990). This primer set was chosen to amplify approximately 600-pb fragments. Reactions were carried out in a 25 µl reaction volume containing 0.5 µM of each primer, 2 µl of 10X buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 1 µl DNA preparation and 1 U of *Taq* polymerase (Invitrogen, USA). Sterile distilled water was used to achieve the final volume of 25 µl. DNA amplifications were performed in an AT-gradient® thermal cycler (Biometra, Germany) using an initial denaturation temperature of 94°C for 4 min, followed by 30 cycles of template denaturation for 30 s at 94°C, primer annealing for 30 s at 58°C and chain elongation for 60 s at 72°C, with a final extension of 7 min at 72°C. The amplified products were verified using 2% agarose gel electrophoresis in 1X Tris acetic acid EDTA (TAE, pH 8.0) buffer, stained with SYBR-safe (Invitrogen, USA) and sequenced at BMR Genomics, DNA Sequencing Service Centre, Padova, Italy. The identity of the sequences was confirmed using BLAST search to known sequences in NCBI Genbank (http://www.ncbi.nlm.nih.gov)

In vitro trial to asses the efficacy of the nematophagous fungi

1. Preparation of eggs

For the assay, approximately 100 egg masses were handpicked from two month-old galled tomato roots in a growing chamber, pooled, and shaken in 4-5 ml of 1.05% NaOCl for 5 min by vigorously drawing the egg suspension in and out of a Pasteur pipette. Debris was allowed to settle from the egg suspension for 30 s. Eggs still in suspension were pipetted onto an autoclaved 500-mesh sieve (pore size 25 µm) and washed four times with sterile distilled water over a 5 min period. Before use, an aliquot of the suspension was removed to estimate the number of eggs per ml.

2. Fungus preparation

Three *Fusarium* isolates (HR4, HR23 and HR57) were kept in test tubes containing 2% PDA, in the dark, at 4°C for 10 days. Culture disks (diameter 4 mm) were extracted from fungi isolates kept in the test tubes and plated into 9-cm diameter Petri dishes containing 20 ml of 2% PDA, and then incubated in the dark, at 25°C for 10 days. After growth of the isolates, new culture disks, 4 mm in diameter, were transferred to 9 cm diameter Petri dishes containing 20 ml of 2% water agar (WA) for 10 days. One hundred eggs of *M. incognita* previously surface sterilized were placed at the edge of the colonies. Observations were made every 24 h under a stereoscopic microscope. In order to estimate fungal ovicidal activity, percentage of egg mortality was calculated by dividing the number of infected eggs to the total eggs multiplied by 100 (Wang *et al.* 2005).

Biocontrol potential of *Fusarium* isolates against *M. incognita*: pot experiment

The antagonistic effects of *Fusarium* spp. against the root-knot nematode were studied in tomato plants. Seeds of tomato cv. 'Rio Grande' susceptible to root-knot nematode, *M. incognita*, were sown in seedbeds filled with an autoclaved mixture (1:1) of sandy loam soil and culture substrate (mixture of black and white peat, clay, 0.5% mineral nitrogen, 90% organic matter and water retention capacity of 60 to 70%). Tomato seedlings of 21 days-old were transplanted in 250 ml plastic pots filled with steam sterilized soil and planted with one tomato seedling/pot. Eggs of *M. incognita* were harvested from infected roots using sodium hypochlorite (Hussey and Barker 1973), suspended in sterilized distilled water and the inoculum was standardized to 1000 eggs/pot. The microconidia of the isolated fungus growing on PDA plates at 25°C for 14 days were harvested by flooding the plates with sterilized distilled water. The resulted suspension was filtered through cheesecloth and then the antagonistic inoculum was adjusted to 1×10^5 spores/ml using a haemocytometer (Bio Kobe). Each pot was watered with 10 ml of the spore suspension. Treatments were arranged as follows: (1) soil infested with *Fusarium* isolates + *M. incognita*; (2) soil infested with each *F. oxysporum* isolate (3) control with *M. incognita* (1000 eggs/pot); (4) untreated control. Each treatment was replicated 7 times (i.e. 7 pots per elementary treatment). The effects of *Fusarium* isolates on *M. incognita* in tomato plants were assessed via several parameters such as plant height, fresh root and shoot weights, egg masses and galls per root system.

Statistical analyses

The data were subjected to analyses of variance (ANOVA) using SPSS 11.0 for Windows. The treatment means were compared by Duncan's multiple range test when *F*-tests were statistically significant at $P < 0.05$.

RESULTS

Identification

Using the universal fungal primers (ITS1/ITS4), PCR products were generated from all of the *Fusarium* species. Sequences of amplified fragments showed 99% identity with *F. oxysporum* ribosomal RNA gene, accession number EU839403.1 and U34571.1 for HR4 and HR57 respectively and 98% identity with *F. chlamydosporum* accession number EU556725.1 for HR23 strain.

Pathogenicity test on *M. incognita* eggs *in vitro*

The ovicidal activity of *F. oxysporum* and *F. chlamydosporum* against *M. incognita* eggs are shown in **Table 1**. Data analysis showed significant difference ($P < 0.05$) for ovicidal activity among the studied isolates. *F. oxysporum* isolate HR57 showed an important parasitic effect of 60, 63 and 70% corresponding to 3, 4, and 7 days post-treatment, respectively. This antagonist had the highest degree of pathogenicity whereas *F. chlamudosporum* (HR23) and *F. oxysporum* (HR4) were less effective. In all the treatments, the percentage of infected eggs gradually increased from the 3rd

Table 1 Means and standard deviation for the ovicidal activity of the *Fusarium oxysporum* isolates (HR4 and HR57) and *F. chlamydosporum* (HR23) and the control group without fungi on eggs of *M. incognita* at 3, 5 and 7 days.

Treatments	Egg mortality (%)		
	3 days	5 days	7 days
F. oxysporum (HR4)	55 ± 7.3 c	59 ± 12 c	61 ± 10.2 b
F. oxysporum (HR57)	60 ± 10.7 c	63 ± 9 c	70 ± 19 c
F. chlamydosporum	46 ± 10.1 b	50 ± 11 b	59 ± 11.2 b
Control	7 ± 7 a	17 ± 7.7 a	17 ± 8 a

The values indicate means ± standard deviation of 5 replicates. Means followed by the same letter within the same column are not significantly different according to Duncan's multiple range test ($P \leq 0.05$)

Table 2 Effect of different *Fusarium* isolates on the root-knot nematode, *M. incognita*, infection and on tomato growth parameters.

Treatments	Plant length (cm)	Fresh root weight (g)	Fresh shoot weight (g)	Egg masses /root system	Galls / root system
F. oxysporum (HR4) + *M. incognita*	35.7 ± 9 bc	8.87 ± 1 ab	10.86 ± 1 ab	95.2 ± 47 ab	126 ± 87 b
F. oxysporum (HR57) + *M. incognita*	39.7 ± 7 c	9.28 ± 1.2 b	12.42 ± 1.9 b	82 ± 34 a	95 ± 54 a
F. chlamydosporum + *M. incognita*	44.71 ± 5 c	9.15 ± 0.8 b	14.52 ± 1.5 b	112 ± 54 ab	133 ± 54 b
F. oxysporum (HR4)	29.37 ± 11 b	6.71 ± 1.5 a	8.57 ± 2.4 a	-	-
F. oxysporum (HR57)	41.28 ± 13 c	8.98 ± 2 ab	11.74 ± 1.5 b	-	-
F. chlamydosporum	30.98 ± 7 b	6 ± 0.5 a	12. 15 ± 1.7 b	-	-
Control with *M. incognita*	2185 ± 5 a	10.63 ± 1.7 b	7.66 ± 1.2 a	141.2 ± 97 b	286 ± 101 c
Control without *M. incognita*	30.42 ± 6 b	7.45 ± 0.7 ab	13.7 ± 1.5 b	-	-

The values indicate means ± standard deviation of 7 replicates. Means followed by the same letter within the same column are not significantly different according to Duncan's multiple range tests ($P \leq 0.05$)

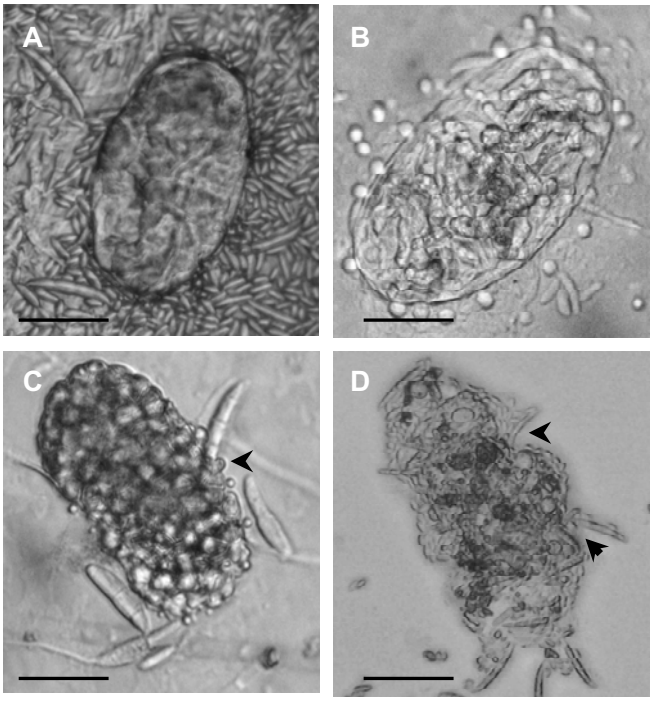

Fig. 1 *Meloidogyne incognita* eggs parasitised by *Fusarium oxysporum* Strain HR57 after 7 days. Condensation of micro- and macroconidia around the *M. incognita* egg (**A**) and morphological degradation of the embryo (**B**). Perforation of the egg shell (arrow heads) and penetration of macroconidia inside the eggs and complete degradation (**C, D**). Scale bars: 20 µm.6.

to the 7th day.

The fungi were also observed as developing in the eggs. The mycelium was proliferating within eggs 72 h after first contact digesting the embryos within eggs, during the early stages of infection. Morphological alteration of embryo and eggshell was observed without hyphal penetration (**Fig. 1A, 1B**), or with internal colonization by micro- and macroconidia (**Fig. 1C, 1D**).

Greenhouse evaluation of biological control activity of *Fusarium* isolates

The results of the pot experiment indicated that application of *Fusarium* isolates to soil had significantly reduced the number of egg masses of *M. incognita* and the root galling on tomato plants. All the antagonistic treatments tested significantly ($P < 0.05$) reduced the number of egg masses and root galls compared with the untreated plants 45 days after inoculation (**Table 2**). The highest reduction in gall formation and egg masses production was obtained with the isolate HR57 of *Fusarium*. Moreover, with *F. chlamydosporum* (HR23*)* and *F. oxysporum* (HR4), galls and egg masses number decreased moderately but significantly.

The effect of all treatments on the fresh weight of tomato roots and shoots was determined at the 45 days after inoculation. The results showed that these growth parameters noted on untreated control tomato plants were significantly ($P < 0.05$) lower than those treated with *Fusarium* spp. isolates.

Table 2 clearly shows that the application of *Fusarium* isolates singly to the soil did not affect plant height and shoots and roots fresh weights compared to the non infected control, indicating that these isolates have no effect on plant growth. The maximum increase in plant height was recorded in infested plants treated with *F. oxysporum* (HR57) which in addition to its nematicidal effect, this strain had enhanced the plant growth.

The most important root weight for this treatment resulted from a better developed root system rather than from an increased weight of galls on roots (**Table 2**).

DISCUSSION

The genus *Fusarium* includes many species with great trophic adaptation. *Fusarium* spp. strains are frequently isolated from nematodes or egg masses (Chen *et al.* 1996; Viaene and Abawi 1998; Verdejo-Lucas *et al*. 2002; Trifonova *et al.* 2009).

Local isolates of *F. oxysporum* were found to reduce root-knot nematode infection of tomato. Gall formation was reduced by 50 and 67% with *F. oxysporum* HR57 and HR4 strains applied to the culture substrate. The reduction in root-knot galling observed in this study is consistent with previously published results obtained with fungal endophytes based on greenhouse experiments on tomato (Hallmann and Sikora 1994; Dababat and Sikora 2007) and other crops (Vu *et al*. 2006; Huang *et al*. 2009; Yan *et al*. 2011). In addition to reducing nematode galling severity, *Fusarium* isolates tested in the present experiment increased the tomato shoot fresh weight compared with non-treated tomato plants except for one *Fusarium* isolate (HR4). This increase may indicate the growth promoting effect of these local strains on tomato.

There are few studies on the mode of action of these fungal species towards plant parasitic nematodes and the mechanisms involved in antagonism are still unclear. However, Sikora (1992) suggested that *Fusarium* strains might produce toxins or compete for space with endoparasitic nematodes and thereby significantly reduce nematode infestation. Secondary metabolites from *F. oxysporum*, were shown to be toxic to *M. incognita* (Hallmann and Sikora 1996), and toxins from various *Fusarium* spp. may affect nematode viability (Ciancio 1995; Anke and Sterner 1997; Nitao *et al*. 2001). However, the mechanism deployed by *Fusarium* in nematode development inhibition appears to be more complex than a toxin-operating system. In fact, based on a split root system experiment, tomato plants were found to be resistant to *M. incognita* infection in the root system half which was not inoculated with *F. oxysporum* showing the involvement of systemic resistance induced by the fungi for the inhibition of nematode infection (Dababat and Sikora 2007).

None of the *Fusarium* isolates tested in this study showed pathogenic symptoms on tomato cv. 'Rio Grande' plants. These results are similar to those of the studies car-

ried by Hallman and Sikora (1994) who demonstrated that *F. oxysporum* isolates which were tested in subsequent greenhouse experiments reduced *M. incognita* populations without adversely affecting plant health, thus indicating that these isolates are promising candidates for the biological control of the tomato root-knot nematode.

In this study, the results demonstrated a correlation between pathogenicity assays conducted *in vitro* and biocontrol efficacy observed in culture substrate. Indeed, *F. oxysporum* HR57 strain which showed an important efficiency in culture substrate showed high percentage of parasitism of *M. incognita* eggs *in vitro* reaching 70%. The ability of *Fusarium* strains to parasitize root-knot nematode eggs *in vitro* has been recorded in previous studies (dos Santos *et al.* 1992; Sun *et al.* 2006). Observation of parasitized eggs on agar showed a morphological alteration of embryo which were digested within eggs, the fungus mechanically penetrated the egg shell through micro- and macrospores, the activity of lytic enzymes appeared involved. In fact, it was reported that eggs disintegration may be solely caused by an enzymatic action (Gortari and Hours 2008) and enzymes secreted by parasitic fungi are known to help colonization of nematode eggs by penetrating the egg shell (Yang *et al.* 2007). However, the contribution of non enzymatic toxins to the success of parasitism has not been studied. As *Fusarium* species are common soil inhabitants, they may interact with root-feeding nematodes either directly or via their secondary metabolites (Nitao *et al.* 2001). Although isolates HR57 and HR23 are promising antagonistic fungi for the control of the root-knot nematodes, but before developing the selected isolates as commercial biological control agents, more extensive tests are necessary to determine their effectiveness on other horticultural crops and under different conditions.

ACKNOWLEDGEMENTS

First author gratefully acknowledges funding by the research unit 04 AGRO 4 (Invertebrates, Microorganisms, Weeds: alternative methods of control) and the Programme ERASMUS MUNDUS Imageen (International Maghreb-Europe Education Network).

REFERENCES

Alabouvette C, Schippers B, Lemanceau P, Bakker PAHM (1998) biological control of Fusarium wilts: towards development of commercial products. In: Boland GJ, Kuykendall LD (Eds) *Plant-microbe Interactions and Biological Control*, Marcel Dekker, New York, pp 15-36

Anke H, Sterner O (1997) Nematicidal metabolites from higher fungi. *Current Organic Chemistry* **1**, 427-440

Ashoub AH, Montasser A, Mourad MH, Gamal M (2009) Impact of some fungi species as biocontrol agent against the root-knot nematode, *Meloidogyne incognita. Australian Journal of Basic and Applied Science* **3**, 3617-3624

Chen S, Dickson DW, Whitty EB (1996) Fungi associated with egg masses of *Meloidogyne incognita* and *M. javanica* in a Florida tobacco field. *Nematropica* **26**, 153-157

Ciancio A (1995) Observations on the nematicidal properties of some mycotoxins. *Fundamental and Applied Nematology* **18**, 451-454

Dababat AA, Sikora RA (2007) Induced resistance by the multualistic endophyte, *Fusarium oxysporum* strain 162, toward *Meloidogyne incognita* on tomato. *Biocontrol Science and Technology* **17**, 969-975

Domsch KH, Gams W, Anderson TH (Eds) (1980) *Compendium of Soil Fungi* (Vol 2), Academic Press Inc., London, New York, 391 pp

dos Santos MA, Ferraz S, Muchovej JJ (1992) Evaluation of 20 species of fungi from Brazil for biocontrol of *Meloidogyne incognita* race-3. *Nematropica* **22**, 183-192

Fravel D, Olivain C, Alabouvette C (2003) *Fusarium oxysporum* and its biocontrol. *New Phytologist* **157**, 493-502

Gortari MC, Hours RA (2008) Fungal chitinases and their biological role in the antagonism onto nematode eggs: A review. *Mycological Progress* **7**, 221-238

Hallmann J, Sikora RA (1994) Occurrence of plant parasitic nematodes and non-pathogenic species of *Fusarium oxysporum* in tomato plants in Kenya and their role as mutualistic synergists for biological control of root-knot nematodes. *International Journal of Pest Management* **40**, 321-325

Hallmann J, Sikora RA (1996) Toxicity of fungal endophyte secondary metabolites to plant parasitic nematodes and soil-borne plant pathogenic fungi. *European Journal of Plant Pathology* **102**, 155-162

Huang TT, Padgham JL, Sikora RA (2009) Biological control of the rice root-knot nematode *Meloidogyne graminicola* on rice, using endophytic and rhizosphere fungi. *International Journal of Pest Management* **55**, 31-36

Hussey RS, Barker KR (1973) A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter* **57**, 1025-1028

Kaur R, Kaur J, Singh RS (2010) Nonpathogenic *Fusarium* as a biological control agent. *Plant Pathology Journal* **9**, 79-91

Minerdi D, Moretti M, Gilardi G, Barberio C, Gullino ML, Garibaldi A (2008) Bacterial ectosymbionts and virulence silencing in a *Fusarium oxysporum* strain. *Environmental Microbiology* **10**, 1725-1741

Niere BI, Speijer PR, Gold CS, Sikora RA (1999) Fungal endophytes from bananas for the biocontrol of *Radopholus similis*. In: Frison EA, Gold CS, Karamura EB, Sikora RA (Eds) *Mobilizing IPM for Sustainable Banana Production in Africa*, INIBAP, Montpellier, France, pp 313-318

Nitao JK, Meyer SLF, Schmidt WF, Fettinger JC, Chitwood DJ (2001) Nematode-antagonistic trichothecenes from *Fusarium equiseti*. *Journal of Chemical Ecology* **27**, 859-869

Olivain C, Humbert C, Nahalkova J, Fatehi J, L'Haridon F, Alabouvette C (2006) Colonization of tomato root by pathogenic and nonpathogenic *Fusarium oxysporum* strains inoculated together and separately into the soil. *Applied and Environmental Microbiology* **72**, 1523-1531

Sikora RA (1992) Management of the antagonistic potential in agricultural ecosystems for the control of plant parasitic nematodes. *Annual Review of Phytopathology* **30**, 245-270

Sikora RA, Fernández E (2005) Nematode parasites of vegetables. In: Luc M, Sikora RA, Bridge J (Eds) *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture* (2nd Edn), CABI Publishing, Wallingford, UK, pp 319-392

Sikora RA, Schafer K, Dababat AA (2007) Modes of action associated with microbially induced *in planta* suppression of plant parasitic nematodes. *The Australian Plant Pathology* **36**, 124-134

Sun MH, Gao L, Shi YX, Li BJ, Liu XZ (2006) Fungi and actinomycetes associated with *Meloidogyne* spp. eggs and females in China and their biocontrol potential. *Journal of Invertebrate Pathology* **93**, 22-28

Trifonova Z, Karadjova J, Georgieva T (2009) Fungal parasites of the root-knot nematodes *Meloidogyne* spp. in Southern Bulgaria. *Estonian Journal of Ecology* **58**, 47-52

Verdejo-Lucas S, Ornat C, Sorribas FJ, Stchiegel A (2002) Species of root-knot nematodes and fungal egg parasites recovered from vegetables in Almeria and Barcelona, Spain. *Journal of Nematology* **34**, 405-408

Viaene N, Abawi GS (1998) Fungi parasitic on egg masses of *Meloidogyne hapla* in organic soils from New York. *Supplement to the Journal of Nematology* **30**, 632-638

Vu T, Hauschild R, Sikora RA (2006) *Fusarium oxysporum* endophytes induced systemic resistance against *Radopholus similis* on banana. *Nematology* **8**, 847-852

Vu TT, Sikora RA, Hauschild R (2004) Effects of endophytic *Fusarium oxysporum* towards *Radopholus similis* activity in absence of banana. *Communications in Agriculture and Applied Biological Science* **69**, 381-386

Wachira PM, Kimenju SA, Okoth, Mibey RK (2009) Stimulation of nematode destroying fungi by organic amendments applied in management of plant parasitic nematode. *Asian Journal of Plant Science* **8**, 153-159

Wang K, Riggs RD, Crippen D (2005) Isolation, selection, and efficacy of *Pochonia chlamydosporia* for control of *Rotylenchulus reniformis* on cotton. *Phytopathology* **95**, 890-893

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) *PCR Protocols: A Guide to Methods and Applications*, Academic Press Inc., New York, pp 315-322

Yan X, Sikora RA, Zheng JW (2011) Potential use of cucumber (*Curcumis sativus* L.) endophytic fungi as seed treatment agents against root-knot nematode *Meloidogyne incognita*. *Journal of Zhejiang University - Science B (Biomedicine and Biotechnology)* **12**, 219-225

Yang J, Tian B, Liang L, Zhang KQ (2007) Extracellular enzymes and the pathogenesis of nematophagous fungi. *Applied Microbiology and Biotechnology* **75**, 21-31