DE GRUYTER
OPEN

Hellenic Plant Protection Journal **9:** 28-34, 2016
DOI 10.1515/hppj-2016-0003

Trichoderma viride and *Pseudomonas fluorescens* CHA0 against *Meloidogyne javanica* in the rhizosphere of tomato plants

A. Saeedizadeh

Summary Root-knot nematodes are among the most important pests that reduce tomato yield in greenhouses and fields in Iran. The scope of this research was to evaluate the antagonistic effect of *Trichoderma viride* and *Pseudomonas fluorescens* CHA0 on the reproduction and galling rate of *Meloidogyne javanica* in tomato roots. A pot experiment was conducted on seedlings of tomato cultivars Bony best, Falat, Mobile and Walter grown in sterilized sandy loam soil. Inocula used for artificial inoculation were 3 J_2/g of soil for the nematode, 1×10^6 spores/ml for the fungus and 1×10^9 cfu/ml for the bacterium. The nematicide RUGBY® 10 G (cadusafos) was used as a reference product at 2g per each pot. Two months after inoculation, the number of knots and egg masses per root in the treatments were (with descending order): control (nematode), nematode+bacterium, nematode+fungus, nematode+fungus+bacterium and nematode+nematicide. The combination fungus+bacterium enhanced the biocontrol effect against *M. javanica* activity as compared to the fungus and bacterium stand-alone treatments except for the cases of the cultivars Mobile and Bonny best in which the effect was similar to the one by the fungus alone. The *fungus* + *bacterium* combined treatment was equally effective to the nematicide treatment for all cultivars. The highest and lowest rate of nematode activity was observed in Walter and Mobile cultivars, respectively.

Additional keywords: biocontrol, fungus, knot, nematode

Introduction

Root-knot nematodes are the most important plant parasitic nematodes in the world, due to the wide host range, worldwide distribution and interactions with phytopathogenic fungi and bacteria (Sasser, 1979). Tomato (*Solanum lycopersicum* L.) is a suitable host for most *Meloidogyne* species. The most important Root-knot nematode species in Iran are *M. incognita* and *M. javanica* with a broad host range and great dispersal, making plant roots more vulnerable to soil pathogens (Hosseini-Nejad and Khan, 2001).

Today, numerous microorganisms have been introduced as antagonists of plant parasitic nematodes (Akhtar and Malik, 2000). Some species of *Trichoderma* have been widely applied as biological control agents against various soil-borne plant pathogens (Whipps, 2001), and several isolates have been successful as biocontrol agents of root-knot nematodes (Sharon *et al.*, 2001). On the other hand, bacteria are the most abundant microorganisms in the soil, and some genera such as *Pasteuria*, *Pseudomonas* and *Bacillus* have also considerable potential as biological control agents against nematodes (Meyer, 2003).

The scope of the study was to evaluate *T. viride* and *P. fluorescens* CHA0 as biocontrol agents against *M. javanica* in four tomato cultivars.

Materials and Methods

Preparation of *M. javanica* inoculum

Plants infected with *M. javanica* were obtained from a population maintained in the glasshouse on tomato plants (cv. Walter) in Varamin, southern Tehran, Iran. Extraction and preparation of the nematode inoculum were applied according to the Hussey and Barker (1973) method using the single egg mass method. According to the morpholog-

Assistant Professor, Department of Plant Protection, Faculty of Agriculture, Shahed University, P.O. Box: 33191-18651, Tehran, Iran
e-mail: ayatsaeed314@gmail.com

ical and morphometrical characteristics of body and perineal pattern, the nematodes were initially identified (Hunt, 1993; Jepson, 1987; Siddiqi, 2000). Then, nematodes were multiplied on the rhizosphere of local tomato cultivars and second stage juveniles (J_2) were finally obtained in the glasshouse. Infected tomato roots bearing large egg masses were incubated in water for three days at 28±2°C and hatched J_2 were collected and counted. Nematode inoculum level for each of the treatments was determined as 3 J_2/g of soil (McClure *et al*., 1973).

Biocontrol of nematode using *T. viride*

Trichoderma viride was obtained from the culture collection of the Department of Plant Pathology, Shahed University, Tehran, Iran. It was cultured on potato dextrose agar (PDA) for 14 days at 25°C. To prepare the inoculum suspension of *T. viride*, about 15ml distilled water were added to the growing colony on PDA medium in a Petri dish. The spores were suspended in distilled water using a sterile glass rod and applied gently on the surface of the colonies. After that, the suspension passed through two layers of sterile net fiber. The number of spores per ml was estimated by a hemocytometer. A spore concentration basis of 1×10^6 spores/ml was adjusted by adding distilled water and suspending (Sahebani and Hadavi, 2008).

Biocontrol of nematode using *P. fluorescens*

Pseudomonas fluorescens CHA0 was obtained from the culture collection of the Department of Plant Pathology, Shahed University, Tehran, Iran. The bacterial inoculum suspension was prepared according to Weller and Cook (1983). A full loop of 48-hour culture of the bacterium on King's medium B (King B) was transferred to a flask containing 100ml King B liquid medium and incubated for 48 hours on shaker (120 RPM) at 27°C. Bacterial suspension was centrifuged for 10 min at 6000 x g, and washed for 2-3 times with a natural salt solution (NaCl 0.14M) to remove residual nutrient medium. Bacterial cells were extracted by recentrifuging and suspending to a solution of 1×10^9 cfu/ml, which was prepared using the standard curve spectrophotometrically in a carboxymethyl cellulose solution (Weller and Cook, 1983).

Plant material and inoculation

Tomato seedlings of four cultivars including Bonny best, Falat, Mobile and Walter were cultivated in sterilized sandy loam soil in the greenhouse. Seedlings were inoculated at the six-leaf stage (aerial parts were intact, and about 20 cm long). Each pot containing one plant, represented one replication and was kept under natural light and 25-27°C. In this experiment, the nematode inoculum was set at 3 J_2/g of soil, the fungus at 1×10^6 spores/ml, the bacterium at 1×10^9 cfu/ml, while 2g of nematicide were added at the appropriate pots. Each pot was filled with 2000g plant substrate. The nematicide and each inoculum were individually applied to each pot in a volume of 5ml suspension in three separate holes around the plants to a depth of 3cm.

Evaluation of *M. javanica* activity on tomato plants

According to the proposed method of Hussey and Johnson (2002) activity of *M. javanica* was evaluated as the number of egg masses and knots per root. The roots of each plant were washed with tap water and drained on blotting paper. To determine the number of egg masses, the roots were divided into 3-4cm parts, then egg masses stained with Floxin solution B (0.15g/l of water), bleached with lactophenole and counted under a dissecting microscope (Hussey and Janssen, 2002; Taylor and Sasser, 1978).

Experimental design and statistical analysis

This experiment was based on a completely randomized design with four tomato cultivars, six treatments for the control of *M. javanica* and five replicates, including: nematode+fungus (NF), nematode+bacterium (NB), nematode+nematicide (NN), nematode+fun-

gus+bacterium (NFB) and control (Cntr). The nematicide RUGBY® 10 G (cadusafos) was used as reference product. Two months after inoculation, the plants were taken from the soil and the number of knots and egg masses per root were counted. The data were then subjected to one way analysis of variance (ANOVA). The means of the treatments were compared using Duncan multiple range test (Steel and Torrie, 1980). All analyses were done by using SAS software version 9.1.

Results

The mean number of knots and egg masses produced by the nematode was significantly different among the studied cultivars (P≤0.0001). The highest number of knots and egg masses belonged to the cultivar Walter while Mobile showed the lowest numbers. In this regard, the cultivars were ranked as Walter> Falat> Bony best> Mobile (Figure 1). Also, the treatments for the control of the nematode had significant effect on the knots and egg masses (P≤0.0001). The highest nematode control based on number of knots was observed when the nematicide was added to the soil (NN) (Figure 2). No statistically significant difference was observed between the NFB and the NN treatments regarding the number of nematode egg masses (Figure 2). In the treatment NFB, the number of nematode knots and egg masses on the roots of different cultivars was lower than in the treatments NF and NB.

The interactive effect of cultivar and treatment was significant (P≤0.0001) suggesting that the cultivars responded differently to the various treatments of nematode control. The lowest number of knots and egg masses (namely the highest nematode control effect) was found at all cultivars under the effect of the nematicide and the NFB treatment (Figure 3). The number of knots and egg masses did not differ between the NFB treatment and the NF treatment for the cultivars Mobile and Bonny best (Figure 3).

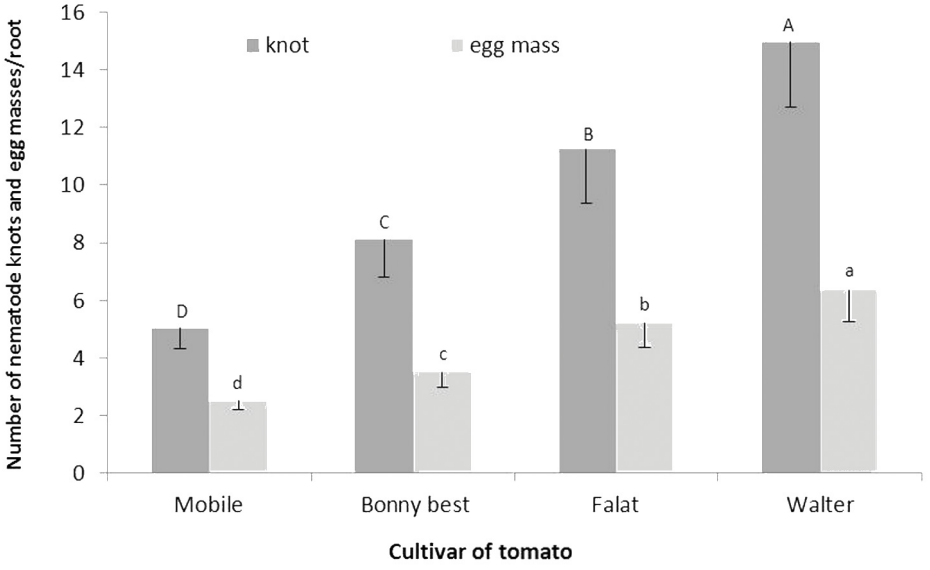

Figure 1. Mean number of nematode knots and egg masses of *Meloidogyne javanica* on the roots of four tomato cultivars (Mobile, Bonny best, Falat, and Walter) treated with *Trichoderma viride* and *Pseudomonas fluorescens* (pooled data of Control, nematode+fungus, nematode+bacterium, nematode+nematicide, nematode+fungus+bacterium). Means with different capital and small letters on the columns (knot and egg mass, respectively) are significantly different (Duncan Test, $P \leq 0.05$; $n = 5$).

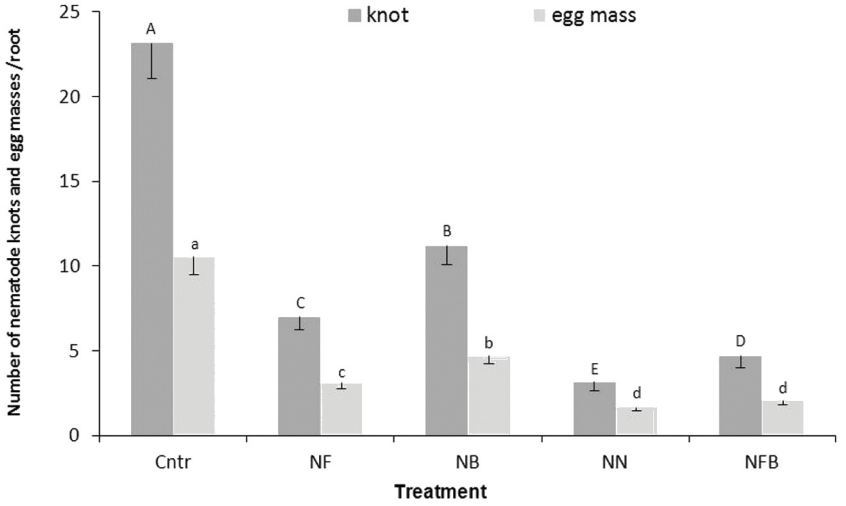

Figure 2. Mean number of nematode knots and egg masses of *Meloidogyne javanica* on roots of four tomato cultivars (pooled data) treated with *Trichoderma viride* and *Pseudomonas fluorescens* (Cntr: Control, NF: nematode+fungus, NB: nematode+bacterium, NN: nematode+nematicide, NFB: nematode+fungus+bacterium). Means with different capital and small letters on the columns (knot and egg mass, respectively) are significantly different (Duncan Test, $P \leq 0.05$; $n = 5$).

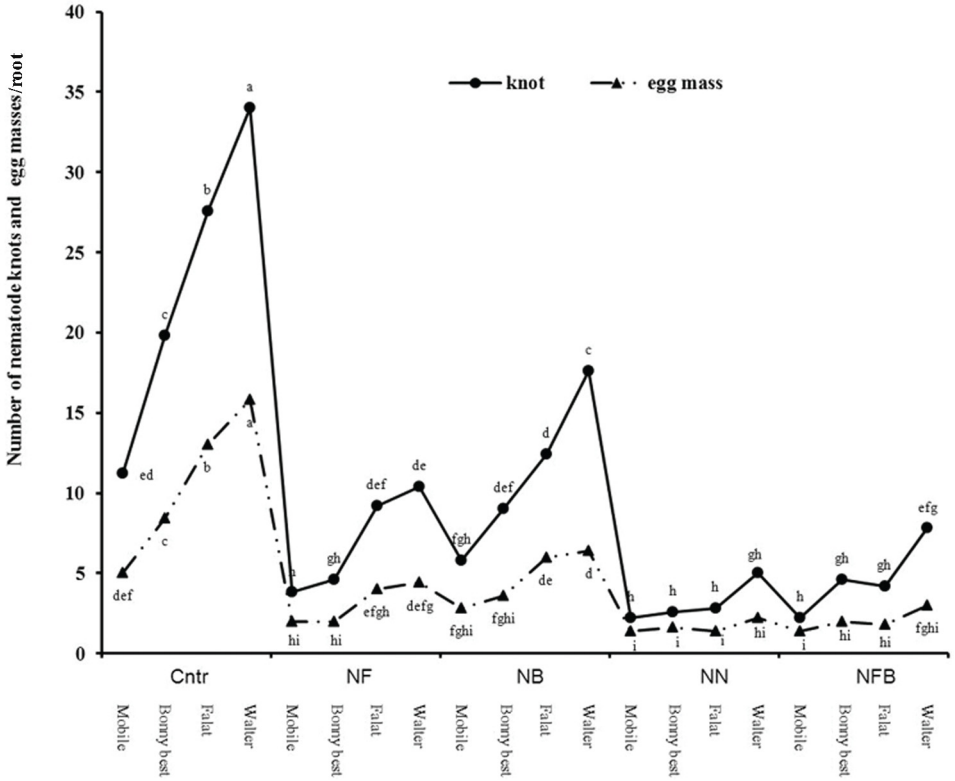

Figure 3. Mean number of nematode knots and egg masses of *Meloidogyne javanica* on roots of four tomato cultivars treated with *Trichoderma viride* and *Pseudomonas fluorescens* (Cntr: Control, NF: nematode+fungus, NB: nematode+bacterium, NN: nematode+nematicide, NFB: nematode+fungus+bacterium). Means with different letters at each of the knot and egg mass lines are significantly different (Duncan Test, $P \leq 0.05$; $n = 5$).

Discussion

Many studies (Ashoub and Amara, 2010; Dababat and Sikora, 2007; Golzari *et al*., 2011; Sahebani and Hadavi, 2008; Sharon *et al*., 2001; Siddiqui and Shaukat, 2003; Siddiqui *et al*., 2005; Siddiqui *et al*., 2004; Zaki *et al*., 2009) have been reported in the field of biological control of plant parasitic nematodes, especially against *Meloidogyne* spp., through the application of several microorganisms, including bacteria and fungi. Natural enemies of nematodes are successful in reducing plant parasitic nematode activity through parasitism, toxins, antibiotics production, enzyme production and competition, inducing systemic resistance in plants and stimulation of plant growth (Tian *et al*., 2007).

In the present study, combination of *T. viride* and *P. fluorescens* CHA0 enhanced the biocontrol effect against *M. javanica* activity as compared to the fungus and bacterium stand alone treatments except for the cases of the cultivars Mobile and Bonny best in which the effect was similar to the one by the fungus alone. Our results also indicated that the *T. viride* and *P. fluorescens* CHA0 combined treatment was equally effective to the nematicide treatment for all cultivars. The combined effect of *P. fluorescens* CHA0 and other fungi species has been evaluated on *Meloidogyne* species control. For example, Siddiqui *et al.* (2004) studied the interaction of six species of *Aspergillus* and bacterial strains *P. fluorescens* CHA0 and *P. fluorescens* CHA0/pME3424 on the control of *M. javanica*, showing that compounds such as methanol and ethyl acetate secreted by *A. niger* enhanced the nematicidal effect of bacterial strains. Zaki *et al.* (2009) investigated the effect of antagonistic fungi and plant growth promoting rhizobacteria (PGPRs) on *M. incognita* in the rhizosphere of tomato. *Aspergillus niger, Paecilomyces lilacinus* and *Penicillium chrysogenum* fungi and *Azetobacter chroococcum, Bacillus subtilis* and *Pseudomonas putida* bacteria were shown to be efficient in diminuting the number of nematode knots and female counts. It was also shown that soil incorporation of *P. lilacinus* had the highest impact on nematode control.

Several studies have indicated that the bacteria have antagonistic effects on the plant parasitic nematodes e.g. *Pseudomonas* on *Meloidogyne* spp. (Kerry, 2000; Jayakumar *et al*., 2002; Siddiqui and Shaukat, 2002, 2003; Andreogloua *et al*., 2003; Siddiqui *et al*., 2005). In recent years, Ashoub and Amara (2010) found that *B. thuringiensis*, *P. fluorescens* RR and *Rhizobium leguminosarum* have been fatal for *M. incognita* juveniles. The latter two species of bacteria also have been efficient in increasing plant growth. Meyer *et al*. (2001) used inoculums of *Burkholderia cepacia* and *T. virens* to manage the activity of *M. incognita* in the rhizosphere of pepper.

Golzari *et al*. (2011) studied the effect of bacterial metabolites of *Pseudomonas aeruginosa* in the control of *M. javanica* on tomato, showing that the 7NSk2, UTPF92 and UTPF86 strains of *P. aeruginosa* produced hydrogen cyanide, protease and salicylic acid, which caused mortality and prevention of nematode egg hatching. In these greenhouse trials, roots of Early Bana tomato cultivar inoculated with the UTPF86 and 7NSK2 strains exhibited the highest plant growth activity and lowest nematode penetration rate, respectively. Sahebani and Hadavi (2008) were able to control *M. javanica* using *T. harzianum* in the greenhouse. This antagonist of the nematode has also been used for the control of *M. incognita* in the rhizosphere of tomato (Al-Fattah *et al*., 2007).

In conclusion, according to our results, the use of a combination of *Trichoderma* spp. and *P. fluorescens* can be more effective against *M. javanica* and will reduce the level of its pathogenic activity. Biocontrol with these two nematode antagonists in a joint application could provide similar results to the use of commercial pesticides at controlled greenhouse conditions.

I wish to acknowledge and appreciate Shahed University, Tehran, Iran for the financial support of this research.

Literature cited

Akhtar, M. and Malik, A. 2000. Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. *Bioresource Technology*, 74: 35-47.

Andreogloua, F.I., Vagelasa, I.K., Woodb, M., Samalievc, H.Y. and Gowena, S.R. 2003. Influence of temperature on the motility of *Pseudomonas oryzi* habitans and control of *Globodera rostochiensis*. *Soil Biology and Biochemistry*, 35: 1095–1101.

Ashoub, A.H. and Amara, M.T. 2010. Biocontrol Activity of Some Bacterial Genera Against Root-Knot nematode, *Meloidogyne incognita. Journal of American Science*, 6(10): 321-328.

Dababat, A. and Sikora, R.A. 2007. Use of *Trichoderma harzianum* and *Trichoderma viride* for the Biological Control of *Meloidogyne incognita* on Tomato. *Jordan Journal of Agricultural Sciences*, 3: 297-309.

Golzari, H., Panjekeh, N., Ahmadzade, M., Salari, M. and Sedaghati, A. 2011. Study the role of fluorescent Pseudomonas bacteria metabolites root-knot nematode *Meloidogyne javanica* on tomatoes in control. *Iranian Journal of Plant Protection Science*, 42(1): 113-124.

Hosseini-Nejad, S.A. and Khan, M.W. 2001. Interaction of root-knot nematode, *Meloidogyne incognita* (race 1), on chickpea cultivars. *Applied Entomology and Phytopathology*, 68: 1-11.

Hunt, D.J. 1993. *Aphelenchida, Longidoridae and Trichodoridae: Their Systematics and Bionomes*. C. A. B. International, Hertfordshine, UK, 352 p.

Hussey, R.S. and Barker, K.R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. Including a new technique. *Plant Disease Reports*, 57: 1025-1028.

Hussey, R.S. and Janssen, G.J.W. 2002. Root-Knot. Nematodes: *Meloidogyne* species. Pp: 43-70. In: J.L. Starr, J. Bridge and R. Cook, (eds). Plant resistance to parasitic nematodes. CAB1 Publishing, Walling ford, UK.

Jayakumar, J., Ramakrishnan, S. and Rajendran, G. 2002. Bio-control of reniform nematode, *Rotylenchulus reniformis* through fluorescent Pseudomonas. *Pesttology*, 26: 45–46.

Jepson SB. 1987. Identification of root–knot nematodes (*Meloidogyne* spp.), C.A.B. International, UK, 265 p.

Kerry, B.R. 2000. Rhizosphere interactions and exploitation of microbial agents for the biological control of plant-parasitic nematodes. *Annual Review of Phytopathology*, 38: 423–441.

McClure, M.A., Kruk, T.H. and Misaghi, I. 1973. A method for obtaining quantities of clean *Meloidogyne* eggs. *Journal of Nematology*, 5: 230.

Meyer, S.L. 2003. United States Department of Agriculture – Agricultural Research Service research programs on microbes for management of plant-parasitic nematodes. *Pest Management Science*, 59: 665–670.

Meyer, S.L.F., Roberts, D.P., Chitwood, D.J., Carta, L.K., Lumsden, R.D. and Mao, W. 2001. Application of *Burkholderia cepacia* and *Trichoderma virens*, alone and in combinations, against *Meloidogyne incognita* on bell pepper. *Nematropica*, 31: 75-86.

Sahebani, N. and Hadavi, N. 2008. Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. *Soil Biology and Biochemistry*, 40: 2016–2020.

Sasser, J.N. 1979. Pathologencity, host ranges and variability in *Meloidogyne* spp. E. Lamberti and C.E. Taylor (eds.). Root–knot nematodes (*Meloidogyne* spp.) systematics, biology and control Academic press, New York, 477 p.

Sharon, E., Bar-Eyal, M., Chet, I., Herrera-Estrella, A., Kleifeld, O. and Spiegel, Y. 2001. Biological Control of the Root-Knot Nematode *Meloidogyne javanica* by *Trichoderma harzianum*. *Phytopathology*, 91(7): 687- 693.

Siddiqi, M.R. 2000. Tylenchida, Parasites of Plants and Insects, 2nd edition. CABI Publishing, Wallingford, Oxon, UK, 833 p.

Siddiqui, I.A. and Shaukat, S.S. 2002. Rhizobacteria-mediated induction of systemic resistance (ISR) in tomato against *Meloidogyne javanica*. *Journal of Phytopathology-phytopathologische Zeitschrift*, 150: 469–473.

Siddiqui, I.A. and Shaukat, S.S. 2003. Suppression of root-knot disease by *Pseudomonas fluorescens* CHA0 in tomato: importance of bacterial secondary metabolite 2,4- diacetylphloroglucinol. *Soil Biology and Biochemistry*, 35: 1615–1623.

Siddiqui, I.A., Haas, D. and Heeb, S. 2005. Extracellular Protease of *Pseudomonas fluorescens* CHA0, a Biocontrol Factor with Activity against the Root-Knot Nematode *Meloidogyne incognita. Applied and Environmental Microbiology*, 5646–5649.

Siddiqui, I.A., Shaukat, S.S. and Khan, A. 2004. Differential impact of some *Aspergillus* species on *Meloidogyne javanica* biocontrol by *Pseudomonas fluorescens* strain CHA0. *Letters of Applied Microbiology*, 39:74–83.

Steel, R.G.D. and Torrie, J.H. 1980. Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc. New York, USA, 481 p.

Taylor, A.L. and Sasser, J.N. 1978. Biology, identification and control of root–knot nematodes (*Meloidogyne* spp.). Coot Publ. Dep. Plant Pathol., North Carolina State university and U.S. Agency Int. Dev. Raleigh, N. C., 111 p.

Tian, B., Yang, J. and Zhang, K. 2007. Bacteria used in the biological control of plant parasitic nematodes: populations, mechanisms of action, and future prospects. *FEMS Microbiology and Ecology*, 61 : 197–213.

Weller, D.M. and Cook, R.J. 1983. Suppression of Take-all of Wheat by Seed Treatments with Fluorescent Pseudomonads. *Phytopathology*, 78: 463-469.

Whipps, J.M. 2001. Microbial Interactions and Biocontrol in the rhizosphere. *Journal of Experimental Botany*, 52:487-511.

Zaki, A., Siddiquia, B. and Futaib, K. 2009. Biocontrol of *Meloidogyne incognita* on tomato using antagonistic fungi, plant-growth-promoting rhizobacteria and cattle manure. *Pest Management Science*, 65: 943–948.

Received: 6 June 2015; Accepted: 13 December 2015

Trichoderma viride και *Pseudomonas fluorescens* CHA0 κατά του κομβονηματώδη *Meloidogyne javanica* στη ριζόσφαιρα φυτών τομάτας

A. Saeedizadeh

Περίληψη Οι κομβονηματώδεις συγκαταλέγονται μεταξύ των πιο σημαντικών εχθρών που προκαλούν μείωση της παραγωγής στην καλλιέργεια θερμοκηπιακής και υπαίθριας τομάτας στο Ιράν. Σκοπός αυτής της μελέτης ήταν η αξιολόγηση της ανταγωνιστικής δράσης του *Trichoderma viride* και του *Pseudomonas fluorescens* CHA0 στην αναπαραγωγή του *Meloidogyne javanica* και το σχηματισμό κόμβων από τον κομβονηματώδη σε ρίζες τομάτας. Πραγματοποιήθηκε πείραμα με σπορόφυτα των ποικιλιών τομάτας Bonny best, Falat, Mobile και Walter grown σε αποστειρωμένο αμμοπηλώδες έδαφος σε γλάστρες. Έγινε τεχνητή μόλυνση με 3 J_2/g εδάφους για τον νηματώδη, 1×10^6 σπόρια/ml για το μύκητα και 1×10^9 cfu/ml για το βακτήριο. Το σκεύασμα RUGBY® 10 G (cadusafos) χρησιμοποιήθηκε σε ποσότητα 2g ανά γλάστρα. Δύο μήνες μετά την μόλυνση, ο αριθμός των κόμβων και των ωόσακκων ανά ρίζα στις διάφορες επεμβάσεις ήταν (κατά φθίνουσα σειρά): μάρτυρας (νηματώδης), νηματώδης+βακτήριο, νηματώδης+μύκητας, νηματώδης+μύκητας+βακτήριο και νηματώδης+νηματωδοκτόνο. Ο συνδυασμός μύκητας+βακτήριο αύξησε την αποτελεσματικότητα της βιολογικής αντιμετώπισης του *M. javanica* σε σύγκριση με τις απλές επεμβάσεις με το μύκητα και το βακτήριο, με εξαίρεση τις ποικιλίες Mobile and Bonny best, στις οποίες η επίδραση ήταν ανάλογη με αυτή του μύκητα. Η συνδυασμένη επέμβαση μύκητας + βακτήριο ήταν εφάμιλλης αποτελεσματικότητας με την επέμβαση του νηματωδοκτόνου για όλες τις ποικιλίες. Η μεγαλύτερη και η μικρότερη δραστηριότητα του νηματώδους παρατηρήθηκε στις ποικιλίες Walter και Mobile, αντίστοιχα.

Hellenic Plant Protection Journal **9:** 28-34, 2016