DOI 10.2478/s11687-013-0121-7

HELMINTHOLOGIA, 50, 2: 132 – 137, 2013

In vitro investigations on the biological control of *Xiphinema index* with *Trichoderma* species

Á. DARAGÓ[1†], M. SZABÓ[2], K. HRÁCS[3], A. TAKÁCS[1‡], P. I. NAGY[3]*

[1]Plant Protection Institute Georgikon Faculty, University of Pannonia, H-8361 Keszthely, P.O. Box 71, Hungary, †E-mail: *agnes.darago@gmail.com*, ‡E-mail: *a-takacs@georgikon.hu*; [2]School of Plant Protection Programme, Szent István University, H-2100 Gödöllő, Páter K str 1, Hungary, E-mail: *szabomarton@rocketmail.com*; [3]Department of Zoology and Animal Ecology, Szent István University, H-2100 Gödöllő, Páter K str 1, Hungary, E-mail: *hracskriszti@gmail.com*, *E-mail: *Nagy.Peter@mkk.szie.hu*

Summary

The application of *Trichoderma* spp. for the suppression of plant-parasitic nematode populations is a promising tool in biological control. Sixteen strains of six *Trichoderma* species (*T. atroviride*, *T. harzianum*, *T. rossicum*, *T. tomentosum*, *T. virens* and *T. asperellum*) were tested *in vitro* in order to identify the most appropriate strains to control the dagger nematode *Xiphinema index*. Mortality assays revealed that the strains of the widely investigated *T. harzianum* species have caused significant reduction of *X. index* populations, although *T. harzianum* strains were not the most efficient among all the tested fungi. Certain *T. virens* and *T. atroviride* strains and *T. rossicum* have triggered faster and higher mortality. Generally, our data indicate that *Trichoderma* species have innate ability to decrease *X. index* population. Furthermore, as we had difficulties with maintaining *X. index in vitro*, we successfully used a newly developed method to keep *X. index* specimens viable during the experiments.

Keywords: mortality; biological control agent; dagger nematodes

Introduction

The number of plant-parasitic nematode species described to date exceeds 4,000. They have a substantial impact on human welfare and economy (Sasser & Freckman, 1987) either by attacking root systems, stems, buds or by acting as vectors of plant viruses. Among other plant-parasitic nematodes, members of family *Longidoridae* are responsible for causing a significant amount of direct and indirect damage in various crop plants (Perry & Moens, 2006). *Xiphinema* is the most diverse genus of the family *Longidoridae*. An indisputably important member of this family is the cosmopolitan species *Xiphinema index*. Besides causing direct damage to the roots, it is capable of transmitting the yellow mosaic (YM) and the vein balding (VB) strains of the grapevine fanleaf virus (GFLV) (Webster, 1972), the most harmful viral disease of grapes worldwide.

Protection against plant-parasitic nematodes is of primary importance in agriculture and horticulture. Despite the propensity of nematicides to be lethal to a broad range of soil organisms, and to induce the development of resistant strains among nematodes, the application of chemicals are the most widely used technique to control plant-parasitic nematodes (Dong & Zhang, 2006; Atreya, 2008; Brun *et al*., 2008).

Certain fungi possess features that make them potential biological control agent (BCA). The application of BCAs like *Trichoderma* spp. is a promising alternative to using chemical pesticides in plant protection (Copping & Menn, 2000). Many strains of *Trichoderma* spp. are able to antagonize plant pathogens, using competition for the substrate, antibiosis, and/or parasitism as main antagonistic strategies (Howell, 2003). *Trichoderma* species are also potential BCAs to control plant-parasitic nematodes (Windham *et al*., 1986; Parvatha *et al*., 1996; Seifullah & Thomas, 1996; Rao *et al*., 1998; Sharon *et al*., 2001; Spiegel *et al*., 2007; Sahebani & Hadavi, 2008; Sharma *et al*., 2009; Yang *et al*., 2010; Affokpon *et al*., 2011; Khan *et al*., 2011; Radwan *et al*., 2012). However, antagonistic potential may vary among species and strains (Bell *et al*., 1982; Schubert *et al*., 2008, Szabo *et al*., 2012). This emphasizes the importance of thorough evaluation before actual field-tests. To clarify the efficacy of selected fungal species and strains, *in vitro* assays were performed and evaluated to determine mortality of *X. index* triggered by *Trichoderma*. Even though *in vitro* assays do not fully represent natural conditions, they still provide information on the most important attributes of the interactions between the two participants.

Materials and methods

The maintenance of fungi and nematode isolation

Strains of *Trichoderma* species (Table 1.) were maintained on potato dextrose agar (PDA; Oxoid) at 25 ºC in a dark chamber. *X. index* Thorne *et* Allen, 1950 females were isolated from soil samples collected in Pécs, southwestern Hungary (UTM: BS80), from the rhizosphere of fig (*Ficus carica* L.). Nematodes were extracted from the soil samples with a Flegg's decantation and sieving method (Flegg, 1967) as described by Brown and Boag (1988), with slightly modified sieve characteristics: first we used a

Table 1. List of *Trichoderma* isolates used in this study

Species	Strain no.	Source	AC number of ITS	ITS genotype
Trichoderma harzianum	SZMC 1600	winter wheat rhizosphere, Algyő, Hungary	DQ345793	2b[1]
Trichoderma harzianum	SZMC 1630	winter wheat rhizosphere, Ruzsa, Hungary	DQ345828	HarII[2]
Trichoderma harzianum	SZMC 2636	winter wheat rhizosphere, Ruzsa, Hungary	DQ345834	HarI[2]
Trichoderma harzianum	SZMC 2637	winter wheat rhizosphere, Tiszasziget, Hungary	DQ345835	2a[1]
Trichoderma harzianum	SZMC 1647	winter wheat rhizosphere, Kunszentmiklós, Hungary	DQ345845	2a[1]
Trichodrema virens	SZMC 1605	winter wheat rhizosphere, Algyő, Hungary	DQ345798	ex-type[3]
Trichodrema virens	SZMC 0931	winter wheat rhizosphere, Ruzsa, Hungary	DQ118083	ex-type[3]
Trichodrema virens	SZMC 1616	winter wheat rhizosphere, Deszk, Hungary	DQ345811	VirI [2]
Trichodrema virens	SZMC 1685	winter wheat rhizosphere, Tiszasziget, Hungary	DQ345883	ex-type[3]
Trichodrema virens	SZMC 1671	winter wheat rhizosphere, Kunszentmiklós, Hungary	DQ345869	VirI[2]
Trichoderma atroviride	SZMC 1609	winter wheat rhizosphere, Deszk, Hungary	DQ345802	MA3643[4]
Trichoderma atroviride	SZMC 1624	winter wheat rhizosphere, Deszk, Hungary	DQ345820	MA3643[4]
Trichoderma atroviride	SZMC 1663	winter wheat rhizosphere, Kunszentmiklós, Hungary	DQ345861	epitype[5]
Trichoderma rossicum	SZMC 1703	winter wheat rhizosphere, Kunszentmiklós, Hungary	DQ345901	MA2995[4]
Trichoderma tomentosum	SZMC 1610	winter wheat rhizosphere, Deszk, Hungary	DQ345804	ND
Trichoderma asperellum	ND	Trifender, Kwizda	ND	ND

SZMC: Microbiological Collection of the University of Szeged; Accession number (AC) of ITS: as listed in GenBank; ITS genotypes are originally described by: [1]Kullnig *et al*., 2000; [2]Kredics *et al*., 2011,; [3]Kullnig *et al*., 2001; [4]Wuczkowski *et al*., 2003; [5]Dodd *et al*., 2003; ND: not determined

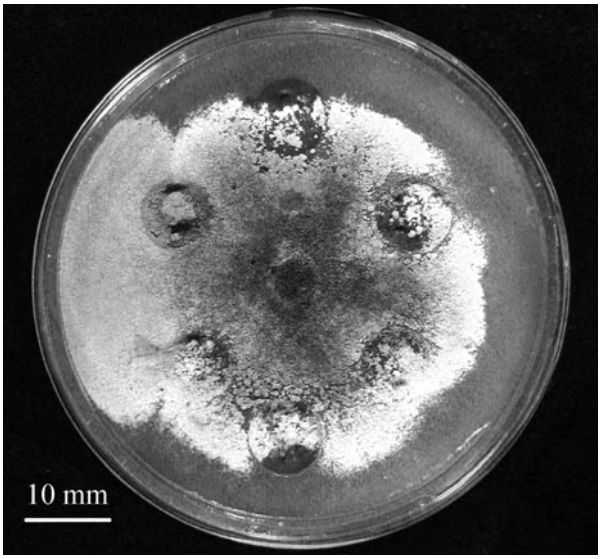

Fig. 1. Experimental Petri dish with soil cores grown over by *T. atroviride* SZMC 1663

coarse sieve of 1000 µm, than the soil sample was washed through a sieve of 180 µm mesh size three times, and then the filtrate was extracted through a sieve of approximately 80 – 90 µm pore size for 12 hours to collect nematodes in a clean final suspension.

Xiphinema mortality assay

To estimate *X. index* mortality triggered by the 16 strains of six *Trichoderma* species, the experiment was run for 8 days. The assays were performed on Minimal Medium (MM) agar plates consisting of 20 g glucose l^{-1}, 5 g $(NH_4)_2SO_4$ l^{-1}, 15 g KH_2PO_4 l^{-1}, 0,6 g $MgSO_4.7H_2O$ l^{-1}, 0,6 g MgCl l^{-1}, 0,6 g $CaCl.2H_2O$ l^{-1}, 5 mg $FeSO_4.7H_2O$ l^{-1}, 1.56 g $MnSO_4.H_2O$ l^{-1}, 1.4 g $ZnSO_4.7H_2O$ l^{-1}, 2 mg $CoCl_2.6H_2O$ l^{-1}. Humus soil with a composition of 80% peat, 15% green compost and 5 % clay was also used for the experiment. It was autoclaved three consecutive days and then left to stand for two weeks prior to use.

To create test dishes, 6 holes (12 mm in diameter) were symmetrically drilled into the MM agar plates and approximately 10 g sterilized soil was placed into the holes. To inoculate the test dishes with fungi, agar plugs from the growing edge of each fungus were removed from plates on which the various fungi were maintained for three days and then plugs were placed in the middle of the experimental MM agar plates (Fig 1). The fungi were allowed to grow until they reached the edge of the holes and then 30 individual *X. index* females were carefully placed into the soil to maintain their viability. Experiments were carried out in triplicates at 25 ºC in a dark chamber. Control experiments were performed under identical conditions, with the ex-

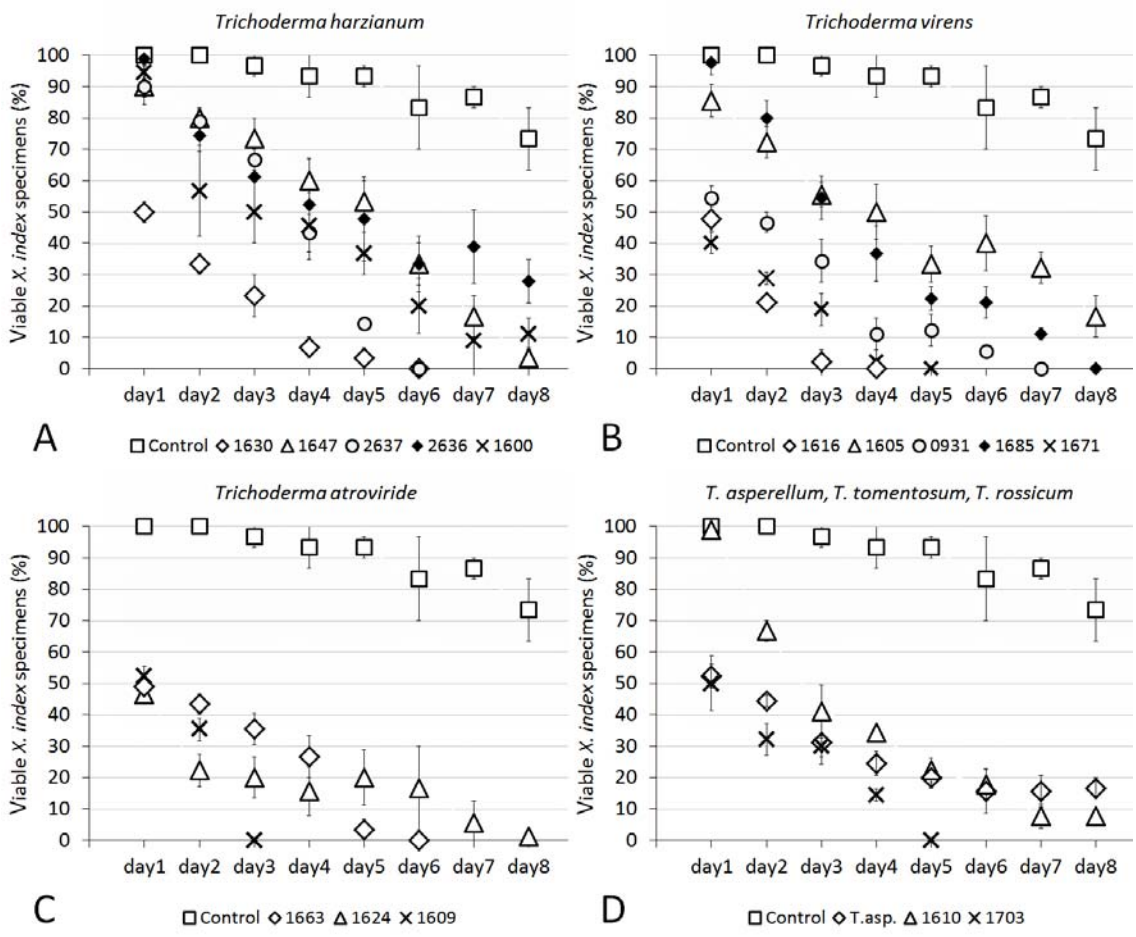

Fig. 2. The effect of different *Trichoderma* strains on *X. index* viability

ception that the Petri dishes contained no fungi. Throughout the experimental period, nematodes were extracted from the soil cores every day. The number of surviving nematodes was evaluated after an extraction time of 12 hours. Dunnett post hoc test was used to determine the effects of *Trichoderma* strains on the survival of *X. index*. The relative *X. index* killing efficiencies of fungal strains compared to the control group were assessed by estimated parameters of generalized linear model model (GLM). Hence, the strain causing the smallest mortality difference was regarded as the least efficient, and the one causing the largest difference was considered the most efficient. All analyses were conducted in R (R Development Core Team, 2011).

Results

Every *Trichoderma* strain had significant effect ($p<1e-10$) on *X. index* survival compared to axenic controls. By the end of the experimental period all *X. index* specimens were killed by 9 out of 16 *Trichoderma* strains: *T. harzianum* (SZMC 1630 and 2637), *T. virens* (SZMC 1616, 1671, 0931 and 1685), *T. atroviride* (SZMC 1663 and 1609) and *T. rossicum* (SZMC 1703).
Regarding the 5 strains of *T. harzianum* (SZMC 1630, 1647, 2637, 2636, and 1600), only SZMC 1630, and 2637 strains caused total *Xiphinema* mortality, while SZMC 2636 triggered the lowest death rate (Fig. 2A; Table 2).
Two strains of *T. virens* (SZMC 1616 and 1671) proved to be the most effective among all examined *Trichoderma* strains (Table 2). They triggered 100 % mortality (Fig. 2B), by the mid-term of the time-course experiment (at day 5).
Furthermore, *T. atroviride* strains (SZMC 1609, 1663 and 1624) were also highly effective (Table 2) and had fast mortal effect, killing approximately half of the *X. index* population 24 hours after inoculation (Fig 2C). On day 2, SZMC 1624 appeared to be the most lethal. However later on, its nematode killing ability fluctuated and remained somewhat less drastic than that of SZMC 1663 and 1609, respectively. SZMC 1624 failed to cause complete mortality of the test organisms by the end of the experimental period. In contrary, no living nematodes could be found in the SZMC 1609 treatment from the third day on.
The species *T. rossicum* SZMC 1703 and *T. asperellum* (Trifender) also eliminated approximately 50 % of the *X. index* specimens as early as the first day (Fig. 2D), while *T. tomentosum* SZMC 1610 remained ineffective in this period. Later on, the mortality caused by *T. tomentosum* SZMC 1610 and *T. asperellum* (Trifender) obtained similar values and remained incomplete by day 8, while *T. rossicum* SZMC 1703 caused a complete mortality by day 5.

Table 2. The order of relative killing efficiency (RKE) of *Trichoderma* isolates against *Xiphinema index* females

Ranking	Species	Strain no.	RKE
1.	*T. virens*	SZMC 1616	-0.78616470
2.	*T. atroviride*	SZMC 1609	-0.69972902
3.	*T. virens*	SZMC 1671	-0.67499440
4.	*T. harzianum*	SZMC 1630	-0.53063244
5.	*T. rossicum*	SZMC 1703	-0.48546547
6.	*T. atroviride*	SZMC 1624	-0.35452940
7.	*T. atroviride*	SZMC 1663	-0.34073456
8.	*T. virens*	SZMC 0931	-0.31345779
9.	*T. asperellum*	Trifender	-0.05660068
10.	*T. harzianum*	SZMC 2637	0.10425142
11.	*T. tomentosum*	SZMC 1610	0.13811580
12.	*T. virens*	SZMC 1685	0.21038886
13.	*T. harzianum*	SZMC 1600	0.23199558
14.	*T. virens*	SZMC 1605	0.43211859
15.	*T. harzianum*	SZMC 1647	0.45595317
16.	*T. harzianum*	SZMC 2636	0.54016307

Discussion

To the best of our knowledge, information on the effect of various *Trichoderma* species on *Xiphinema index* has not been available to date. Among the *Trichoderma* species examined with respect to their ability to kill plant-parasitic nematodes, mostly *T. harzianum* has been tested (Sharon *et al*., 2001). Furthermore, many publications report interactions between plant-parasitic nematodes and soil fungi; however, most of the studies were performed on *Meloidogyne* species (Rao *et al*., 1997). Among other *Trichoderma* species of this study, *T. harzianum* strains have caused significant reduction of *X. index* populations, although they did not prove to be the only efficient strains of all the tested fungal species. In general, strains of *T. virens*, *T. atroviride* and *T. rossicum* have triggered faster and more complete mortality than strains of *T. harzianum*. The evaluation of the mortality assays indicated that *Trichoderma* species have innate ability to decrease *X. index* population *in vitro*. Additionally, we designed and successfully used a new method to keep *X. index* specimens viable during the experiments. The presented data contribute to the further development of biological control agents against *Xiphinema* species.

Acknowledgements

The first author is grateful to late Dr. Miklós Nádasy who piqued her interest in nematology, with special respect to biological control of plant nematodes. May this paper be dedicated to his memory. The second author expresses his gratitude to Dr. Csaba Fekete (Department of General and

Environmental Microbiology, University of Pécs) for the stimulating discussions. We are thankful to Dr. László Kredics (Faculty of Sciences Department of Microbiology, University of Szeged) for providing the *Trichoderma* isolates. The second author was financially supported by the DDKKK Innovation Non-profit Inc. (KOBIOCHG, TECH_09_A4-2009-0124 and GOP-1.1.2-07/1-2008-0008). Part of the work has been supported by KTIA_AIK_12-1-2012-0012.

References

AFFOKPON, A., COYNE, D. L., HTAY, C. C., AGBÉDÉ, R. D., LAWOUIN, L., COOSEMANS, J. (2011): Biocontrol potential of native *Trichoderma* isolates against root-knot nematodes in West African vegetable production systems. *Soil Biol. Biochem*., 43: 600 – 608. DOI: 10.1016/j.soilbio.2010.11.029

ATREYA, K. (2008): Health costs from short-term exposure to pesticides in Nepal. *Soc. Sci. Med*., 67: 511 – 519. DOI: 10.1016/j.socscimed.2008.04.005.

BELL, D. K., WELLS, H. D., MARKHAM, C. R. (1982): In vitro antagonism of *Trichoderma* species against six fungal plant pathogens. *Phytopathology*, 72: 379 – 382

BROWN, D. J. F., BOAG, B. (1988): An examination of methods used to extract virus-vector nematodes (Nematoda: *Longidoridae* and *Trichodoridae*) from soil samples. *Nematol. Mediterr*., 16: 93 – 99

BRUN, G. L., MACDONALD, R. M, VERGE, J., AUBÉ, J. (2008): Long-term atmospheric deposition of current-use and banned pesticides in Atlantic Canada; 1980 – 2000. *Chemosphere*, 71: 314 – 327. DOI: 10.1016/j.chemosphere.2007.09.003

COPPING, L. G., MENN, J. J. (2000): Biopesticides: a review of their action, applications and efficacy. *Pest Manag. Sci*., 56: 651 – 676. DOI: 10.1002/1526-4998(200008)56:8<651::AID-PS201>3.0.CO;2-U

DODD, S. L., LIECKFELDT, E., SAMUELS, G. J. (2003): *Hypocrea atroviridis* sp. nov., the teleomorph of *Trichoderma atroviride*. *Mycologia*, 95: 27 – 40

DONG, L. Q., ZHANG, K. Q. (2006): Microbial control of plant-parasitic nematodes: a five-party interaction. *Plant Soil*, 288: 31 – 45. DOI: 10.1007/s11104-006-9009-3

FLEGG, J. J. M. (1967): Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb's decanting and sieving technique. *Ann. Appl. Biol*., 60: 429 – 437

HOWELL, C. R. (2003): Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. *Plant Dis*., 87: 4 – 10

KREDICS, L., LÁDAY, M., KÖRMÖCZI, P., MANCZINGER, L., RÁKHELY, G., VÁGVÖLGYI, C., SZEKERES, A. (2011): Genetic and biochemical diversity among *Trichoderma* isolates in soil samples from winter wheat fields of the Pannonian Plain. *Acta Biol. Szeged.* (in press)

KHAN, M. R., HAGUE, Z. (2011): Soil application of *Pseudomonas fluorescens* and *Trichoderma harzianum* reduces root-knot nematode, *Meloidogyine incognita*, on tobacco. *Phytopathol. Mediterr*., 50: 257 – 266

KULLNIG, C. M., SZAKACS, G., KUBICEK, C. P. (2000): Molecular identification of *Trichoderma* species from Russia, Siberia and the Himalaya. *Mycol. Res*., 104: 1117 – 1125. DOI: 10.1017/S0953756200002604

KULLNIG, C. M., KRUPICA, T., WOO, S. L., MACH, R. L., REY, M., BENÍTEZ, T., LORITO, M., KUBICEK, C. P. (2001): Confusion abounds over identities of *Trichoderma* biocontrol isolates. *Mycol. Res*., 105: 770 – 772. DOI: 10.1017/S0953756201229967.

PARVATHA, R. P., RAO, M. S., NAGESH, M. (1996): Management of citrus nematode, *Tylenchulus semipenetrans*, by integration of *Trichoderma harzianum* with oil cakes. Nematologia Mediterrannea, 24: 265 – 267

PERRY, R. N., MOENS, M. (2006): *Plant Nematology*. Wallingford, UK, CABI Publishing, pp. 4 – 166

R DEVELOPMENT CORE TEAM (2011): R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/.

RADWAN, M. A., FARRAG, S. A. A., ABU-ELAMAYEM, M. M. (2012): Biological control of the root-knot nematode, *Meloidogyne incognita* on tomato using bioproducts of microbial origin. *Appl. Soil Ecol*., 56: 58 – 62. DOI: 10.1016/j.apsoil.2012.02.008

RAO, M. S., REDDY, P. P., NAGESH, M. (1997): Management of root-knot nematode, *Meloidogyne incognita* on tomato by integration of *Trichoderma harzianum* with neem cake. *Z. Pflanzenkr. Pflanzenschutz*, 104: 423 – 425

RAO, M. S., REDDY, P. P., NAGESH, M. (1998): Evaluation of plant based formulations of *Trichoderma harzianum* for management of *Meloidogyne incognita* on egg plant. *Nematol. Mediterr*., 26: 59 – 62

SAHEBANI, N., HADAVI, N. (2008): Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. *Soil Biol. Biochem*., 40: 2016 – 2020. DOI: 10.1016/j.soilbio.2008.03.011

SASSER, J. N., FRECKMAN, D. W. (1987): A world perspective on nematology: the role of the society. In: VEECH, J. A., DICKSON, D. W. (Eds) Vistas on Nematology: Society of Nematologists, Inc. Hyattsville, Maryland., USA, pp. 7 – 14

SCHUBERT, M., FINK, S., SCHWARZE, F. W. M. R. (2008): In vitro screening of an antagonistic *Trichoderma* strain against wood decay fungi. *Arboric. Journal*, 31: 227 – 248. DOI: 10.1080/03071375.2008.9747541

SEIFULLAH, P., THOMAS, B. J. (1996): Studies on the parasitism of *Globodera rostochiensis* by *Trichoderma harzianum* using low temperature scanning electron microscopy. *Afro-Asian J. Nematol*., 6: 117 – 122

SHARMA, P., PANDEY, R. (2009): Biological control of root-knot nematode; *Meloidogyne incognita* in the medicinal plant; *Withania somnifera* and the effect of biocontrol agents on plant growth. *Afr. J. Agric. Res*., 4: 564 – 567

SHARON, E., BAR EYAL, M., CHET, I., HERRERA ESTRELLA, A., KLEIFELD, O., SPIEGEL, Y. (2001): Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. *Phytopathology*, 91: 687 – 693. DOI: 10.1094/PHYTO.2001.91.7.687

SPIEGEL, Y., SHARON, E., BAR-EYAL, M., VAN ASSCHE, A., VAN KERCKHOVE, S., VANACHTER, A., VITERBO, A., CHET, I. (2007): Evaluation and mode of action of *Trichoderma* isolates as biocontrol agents against plant-parasitic nematodes. In *Proceedings of IOBC/WPRS Meeting*, Spa, Belgium, IOBC/WPRS Bulletin, 30: 129 – 133

SZABÓ, M., CSEPREGI, K., GÁLBER, M., VIRÁNYI, F., FEKETE, CS. (2012): Control plant-parasitic nematodes with *Trichoderma* species and nematode-trapping fungi: The role of *chi18-5* and *chi18-12* genes in nematode egg-parasitism. *Biol. Control*, 63: 121 – 128. DOI: 10.1016/j.biocontrol.2012.06.013

WEBSTER, M. J. (1972): *Economic Nematology*. London, UK, Academic Press, pp. 339 – 376

WINDHAM, G. L., WINDHAM, M. T., WILLIAMS, W. P. (1986): Effect of *Trichoderma* spp. on maize growth and *Meloidogyne arenaria* reproduction. *Plant Dis. Report*., 73: 493 – 494

WUCZKOWSKI, M., DRUZHININA, I., GHERBAWY, Y., KLUG, B., PRILLINGER, H., KUBICEK, C. P. (2003): Species pattern and genetic diversity of *Trichoderma* in a mid-European, primeval floodplain-forest. *Microbiol. Res*., 158: 125 – 133. DOI: 10.1078/0944-5013-00193

YANG, Z. S., LI, G. H., ZHAO, P. J., ZHENG, X., LUO, S. L., LI, L., NIU, X. M., ZHANG, K. Q. (2010): Nematicidal activity of *Trichoderma* spp. and isolation of an active compound. *World J. Microbiol. Biotechnol*., 26: 2297 – 2302. DOI: 10.1007/s11274-010-0410-y

RECEIVED FEBRUARY 14, 2013

ACCEPTED MARCH 8, 2013