

Efficacy of *Trichoderma* spp. against Common Fungal Pathogens

Sonja Tančić Živanov* · Radivoje Jevtić · Mirjana Lalošević · Dalibor Živanov ·
Sladana Medić-Pap · Vesna Župunski

Institute of Field and Vegetable Crops, 21000 Novi Sad, Maksima Gorkog 30, Serbia

Summary: Nowadays, organic food production demands more environmental friendly control of plant diseases. Biocontrol based on *Trichoderma* spp. usage is a promising due to *Trichoderma* is aggressive to broad range of phytopathogenic fungi. Given that, the aim of this study was to test *in vitro* antagonistic ability of ten native Serbian *Trichoderma* strains to ten common fungal pathogens. Study confirmed that *Trichoderma* spp. inhibits radial growth of *Ascochyta pinodella* (76.9%), *A. pinodes* (60.0%), *A. pisi* (68.5%), *Fusarium graminearum* (71.1%), *F. proliferatum* (63.9%), *F. verticillioides* (62.6%), *F. oxysporum* (63.9%), *Macrophomina phaseolina* (63.8%), and *Pyrenophora teres* (83.9%). These are first reports of *Trichoderma* spp. *in vitro* efficacy against *A. pisi*, *A. pinodes*, *A. pinodella* and *P. teres*. The lowest inhibitory effect was registered in dual cultures with *Sclerotinia sclerotiorum* - 52.2%.

Key words: *Ascochyta* spp., *Fusarium* spp., *M. phaseolina*, *P. teres*, *S. sclerotiorum*, *Trichoderma* spp.

Introduction

The most common control methods of plant diseases that have been used so far are fungicide application in combination with host resistance to major pathogens. Nowadays, organic food production is in progress and demands more environmental friendly methods for dealing with devastating cosmopolitan pathogens such as *Ascochyta pinodella*, *A. pinodes*, *A. pisi*, *Fusarium graminearum*, *F. proliferatum*, *F. verticillioides*, *F. oxysporum*, *Macrophomina phaseolina*, *Pyrenophora teres*, and *Sclerotinia sclerotiorum*.

Farmers around the world are familiar with cosmopolitan aggressive pathogens, above all with *Sclerotinia sclerotiorum* (Lib.) de Bary and *Fusarium* species. *S. sclerotiorum* is known as a threat to numerous crops, such as: sunflower, soybean, oilseed rape, edible dry bean, chickpea, peanut, field pea, lentils and various vegetables as well (Bolton et al., 2006). *S. sclerotiorum* causes white rot of plants root, stem and fruit or pod, ending with complete plant wilting and rotting if infection starts early. In Serbia, Sclerotinia wilt is the most common form of Sclerotinia disease. Its average frequency on sunflower in Serbia is about 15-20%, but

in some years the frequency can reach even around 50% (Marić et al., 1988).

Taxonomy of *Fusarium* species is complex, and up to 1000 species have been identified at times. Out of 101 most economically important plants, 81 have at least one plant associated with *Fusarium* disease (Moretti, 2009). Generally, *Fusarium* species can cause root, stem and ear rot which causes yield reductions estimated between 10% and 30% in Europe (Logrieco et al., 2002). Species of *Liseola* section, where *F. verticillioides* Sheldon and *F. proliferatum* (Matsushima Nirenberg) belong, are common in temperate regions mainly as pathogens of maize (Logrieco et al., 2002;), wheat (Furlong et al., 2005), barley (Bottalico, 1998), oat (Bottalico & Perrone, 2002), hops (Stanković et al., 2008), sorghum (da Silva et al., 2006), asparagus (Elmer et al., 1999), etc. Maize, wheat and barley constitute almost 80% of European grain production, and another important pathogen of those crops, *F. graminearum*, is registered all over the world (Varga et al., 2002; Tomczak et al., 2002; Doohan et al., 2003; Tančić et al., 2009). Beside these crops, *F. graminearum* has been reported as pathogen of soybean (Dulić, 1987), rice (Nyvall et al., 1999), sorghum (Leslie et al., 1990), millet (Onyike et al., 1991) etc. *F. oxysporum* is also cosmopolitan species which can be isolated from deserts and tropical soils as well as from Arctic soils (Leslie & Summerell, 2006). It is known as an important vascular wilt pathogen on numerous plant species worldwide, mainly vegetable crops of *Solanaceae* family (tomatoes, peppers, potatoes, eggplant), but also alfa-alfa (Krnjaja

Corresponding author:
sonja.tancic@jifvns.ns.ac.rs

Acknowledgements:
This research was part of the project TR31025 supported by Ministry of Education, Science and Technological Development of Republic of Serbia.

et al., 2007), sunflower (Tančić et al., 2012), soybean (Jasnić et al., 2005), sugarcane, legumes, lettuce, watermelon etc. (http://eol.org/pages/187980/hierarchy_entries/57331216/overview).

Another important pathogen common in mixed infections with *F. oxysporum* on sunflower and maize is *M. phaseolina* Tassi (Goid.) (Bhatti and Kraft, 1992). *M. phaseolina* is a soil borne pathogen with a wide host range which causes disease (charcoal rot) on more than 500 cultivated and wild plant species (<https://projects.ncsu.edu/cals/course/pp728/Macrophomina/host.htm>). This pathogen is prevalent in regions with arid subtropical and tropical climates, but it can be also registered in moderate climates when high temperature and dry conditions occurs. It is estimated that charcoal rot affects the crop throughout the world reducing seed yields by 20-36% (Jiménez-Díaz et al., 1983).

P. teres, causal agent of net blotch of barley, is a major disease worldwide that causes yield losses ranged from 10% to 40% (Ma et al., 2004). It is most severe in temperate regions of high rainfall and humidity, but epidemics can occur in low rainfall areas as well (Steffenson & Webster, 1992). There are two types of symptoms caused by this pathogen: net-type lesions caused by *P. teres* f. *teres* and spot-type lesions caused by *P. teres* f. *maculata*. In recent years, epidemics of *P. teres* f. *maculata* have occurred throughout the world (McLean et al., 2010), reducing grain quality parameters such as 1,000-grain-weight by up to 19% (Jayasena et al., 2007).

Ascochyta blight is one of the most economically important diseases of pea and other legumes such as: faba bean, chick pea, grass pea, vetches, etc. (Peever et al., 2007; Barilli, 2016). Pathogens from this complex occur in all areas of the world where legumes are grown. Under favorable weather conditions, the average yield loss caused by Ascochyta blight is estimated above 50% (McDonald and Peck, 2009). Ascochyta blight of pea, is caused by a complex of three pathogens: *A. pisi* Lib., *A. pinodes*

(teleomorph: *Mycosphaerella pinodes* (Berk. & Blox.) Vestergr.) and *A. pinodella* (L.K. Jones). Among them, *A. pinodes* is the most important one (Ali et al., 1978) and can cause yield reduction between 50 to 75 % (Wallen, 1974).

Biocontrol of such devastating pathogens is a big challenge, but there is a great potential in *Trichoderma* spp. usage in these purposes. Presence of great variety of cell-wall degrading enzymes and secondary metabolites makes *Trichoderma* strongly aggressive to broad range of phytopathogenic fungi (Vinale et al., 2008). When direct confrontation with pathogen occurs, the main biocontrol mechanisms of *Trichoderma* species are mycoparasitism and antibiosis (Howel, 2003). *Trichoderma* species are mainly soil fungi found in agricultural soils, native prairie, forests, salt marsh, desert soils of all climatic zones, but also in dead plant material, living roots of various plant species, seeds, lake water and air (Monte, 2001). World-wide distribution, fast growth and high spore production make those species easy to find and isolate. After all, it should be underlined that not all *Trichoderma* strains are effective, most of them are not, and some may even be phytotoxic or pathogenic (Menzies, 1993), so strain selection is of crucial importance.

Given that, the aim of this study was to test antagonistic ability of native Serbian *Trichoderma* isolates to ten common fungal pathogens (*A. pinodella*, *A. pinodes*, *A. pisi*, *F. graminearum*, *F. proliferatum*, *F. verticillioides*, *F. oxysporum*, *M. phaseolina*, *P. teres*, and *S. sclerotiorum*).

Material and Methods

Collection of isolates

Samples of common pathogens were obtained from naturally infected plants from four different host plants, while *Trichoderma* spp. isolates were obtained from soil samples originating from different localities in Serbia (Table 1). The collection was formed throughout the period of 2009-2015.

Table 1. Tested isolates data – isolate's code, origin, host plant and year of isolation

Isolate code	Species	Origin	Host Plant / Substrate	Year of Isolation
ScSc	<i>S. sclerotiorum</i>	Rimski Šančevi	sunflower	2010
MpH	<i>M. phaseolina</i>	Bačka Topola	sunflower	2009
FOx	<i>F. oxysporum</i>	Deliblato	sunflower	2009
FV	<i>F. verticillioides</i>	Rimski Šančevi	maize	2015
FP	<i>F. proliferatum</i>	Rimski Šančevi	maize	2015
FG	<i>F. graminearum</i>	Rimski Šančevi	maize	2014
PT	<i>P. teres</i>	Rimski Šančevi	barley	2014
APs	<i>A. pisi</i>	Rimski Šančevo	Field pea	2015
APn	<i>A. pinodes</i>	Novi Bečej	Field pea	2014
APi	<i>A. pinodella</i>	Lukićevo	Field pea	2014
TR1	<i>Trichoderma</i> sp.	Rimski Šančevi	Soil sample	2011
TR2	<i>Trichoderma</i> sp.	Kuršumlija	Soil sample	2012
TR3	<i>Trichoderma</i> sp.	Rakovački potok	Soil sample	2012
TR4	<i>Trichoderma</i> sp.	Rimski Šančevi	Soil sample	2012
TR5	<i>Trichoderma</i> sp.	Beležiš	Soil sample	2012
TR6	<i>Trichoderma</i> sp.	Surduk	Soil sample	2012
TR7	<i>Trichoderma</i> sp.	Beležiš	Soil sample	2012
TR8	<i>Trichoderma</i> sp.	Surduk	Soil sample	2012
TR9	<i>Trichoderma</i> sp.	Surduk	Soil sample	2012
TR10	<i>Trichoderma</i> sp.	Surduk	Soil sample	2012

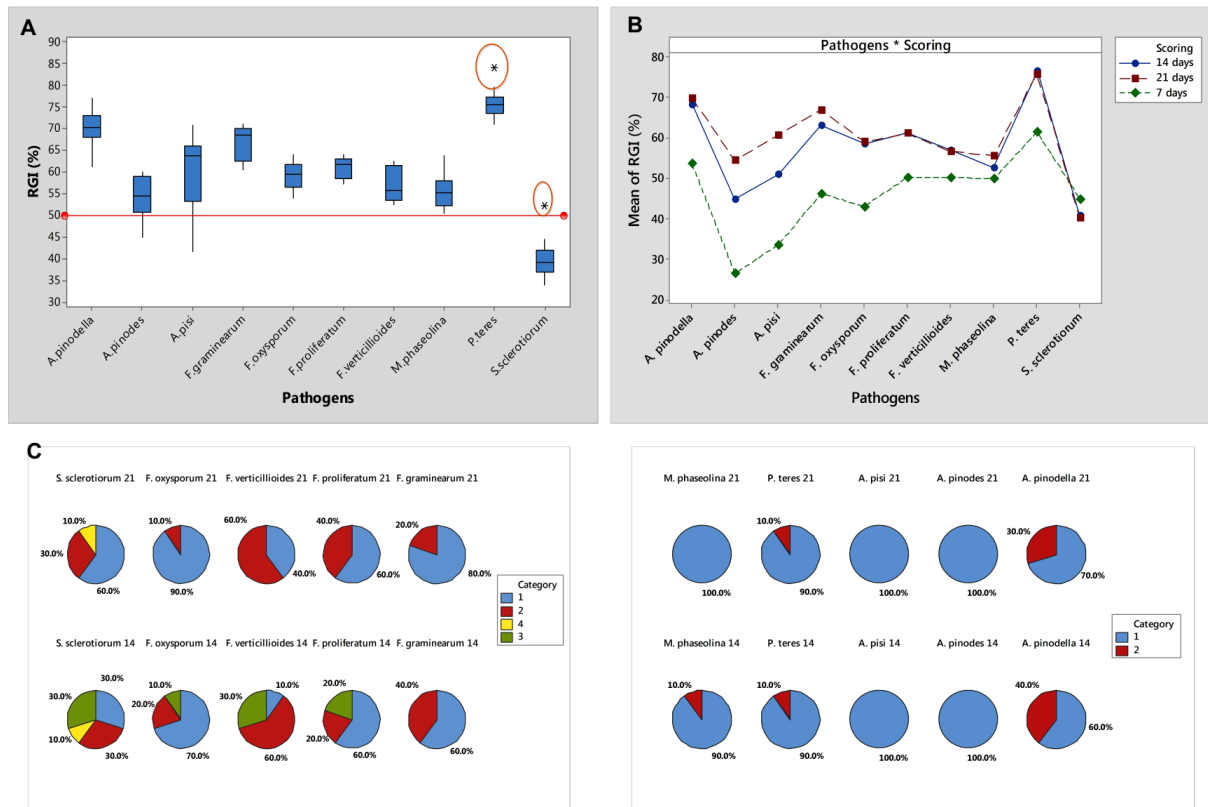


Figure 1. Antagonistic ability of *Trichoderma* spp. isolates against ten common pathogens measured by: (A) RGI on 21st day of incubation *in vitro*, (B) RGI on 7th, 14th and 21st day of incubation *in vitro*, (C) Bell's graduation on 14th and 21st day of incubation *in vitro*

Isolation of the fungus

Samples of maize ears, barley leaves, sunflower stems and heads, and field pea leaves and pods were collected from randomly chosen plants with typical disease symptoms. Pieces of infected tissue were placed on potato dextrose agar (PDA) medium amended with streptomycin and after seven days colonies with morphology and growth characteristics of *Fusarium* species, *S. sclerotiorum*, *M. phaseolina*, *P. teres* and *Ascochyta* species were selected for further purification. Suspensions of conidia or microsclerotia were dispersed on water-agar (WA) plates, and after 24 hours pure culture of each isolate were made by single spore or hyphal tip transfer technique (Leslie & Summerell, 2006). These purified isolates were used for further analyses.

Isolation of biocontrol agent

Trichoderma spp. isolates were obtained from soil samples originating from different soil types and localities in Serbia, mainly from the Vojvodina Province. The representative soil samples were taken from the surface layer (0–20 cm depth) using soil probe. Selective water agar (WA) media amended with streptomycin was used to isolate *Trichoderma* spp. from soil samples by particle-plating method. Total of 20 soil pieces per sample were analysed seven days after incubation at room temperature. The emerging fungal colonies were

observed under microscope, and transferred on suitable media for further analyses of morphological characters. For further research, all *Trichoderma* isolates were refined to single-spore isolates according to Leslie & Summerell (2006).

Dual Culture Test

Dual culture test was used for screening of ten *Trichoderma* isolates antagonistic effect on ten different pathogens *in vitro*. Each *Trichoderma* isolate plug of 7 days old culture (5 mm²) was confronted with the pathogens isolate plug in 90 mm Petri plates at the 60 mm distance on PDA in four replicates. Antagonistic abilities of *Trichoderma* isolates were registered periodically on 7th, 14th, and 21st day of incubation in dark at 25°C. After 21st day of incubation, Radial Growth Inhibition (RGI) was calculated according to Rodriguez et al. (2000). Additionally, evaluation according to Bell was included (de Figueirêdo et al., 2010): 1 – *Trichoderma* sp. grows and covers completely all colonies of phytopathogen surface; 2 – *Trichoderma* sp. grows and covers 2/3 of medium surface; 3 – antagonist and phytopathogen colonize each one half of the medium surface and none seems to dominate each other; 4 – phytopathogen colonizes 2/3 of surface medium; 5 – phytopathogen grows and covers completely all colonies of *Trichoderma* sp. and the medium surface.

Statistical analysis

Factors that significantly influenced RGI were estimated using General Linear Model. For all analysis software Minitab 17 (trial version) was used.

Results and Discussion

Although *Trichoderma* species are mainly registered as an effective antagonists of many soil-borne pathogens on different crops this study shown that isolates of *Trichoderma* spp. which possess antagonistic ability against some pathogens are not effective against the others (Figure 1A). The significant influence of *Trichoderma* isolates and fungal species on RGI was confirmed with $P=0.000$ and $P=0.000$, respectively. *Trichoderma* spp. isolates were considered as effective when RGI exceeded 50%. In addition, significant influence of scoring intervals ($P=0.000$) as well as interaction between pathogens and scoring intervals ($P=0.000$) on RGI was confirmed (Figure 1B). In order to have a better insight in antagonist–pathogen interactions Bell's graduation was used (Figure 1C).

In this study only 1 out of 10 isolates was proven to be effective in *S. sclerotiorum* mycelial growth inhibition (RGI above 50%). This was consistent with studies of Joshi et al. (2010) who reported that only 10% of analysed *Trichoderma* isolates originating from Western Himalayas were effective in inhibition of mycelial growth of *S. sclerotiorum*. According to Joshi et al. (2010) inhibitory effect of tested *Trichoderma* spp. isolates is reported to be much higher than in this study. In contrast, *Trichoderma* isolates tested herein were highly effective against *P. teres* reaching RGI of 83.9% (Figure 1A). Generally, rapid growth of *Trichoderma* spp. isolates gives them important advantage over *P. teres* in the competition for space and nutrients, but more investigation should be done in order to explain the exact mechanism of inhibitory effect of *Trichoderma* isolates. Ali-Haimoudet al. (1993) reported that *Trichoderma* spp. were efficient antagonists of sclerotoid organ formation of *Pyrenophora* (syn. *Drechslera*) *teres* on barley.

In addition, results of this study are first report of *Trichoderma* spp. inhibiting activity on mycelial growth of *A. pisi*, *A. pinodes* and *A. pinodella*. So far, *Trichoderma* spp. antagonistic activity was mainly tested on *Ascochyta reibei*, agent responsible for the anthracnose of chick-pea. Benzohraet al. (2011) observed inhibiting action of *T. harzianum* on the mycelial growth of *A. reibei*, followed by a complete stop of growth after the 7th day and RGI varied from 20-70%, which is similar to results presented in this work.

In this study, *Trichoderma* spp. isolates tested in dual culture with *M. phaseolina* expressed RGI between 50.6 – 63.8% which is a bit more effective than RGI of 33.2 – 61.4% obtained in dual culture test reported by Singh et al. (2008).

Biocontrol of *Fusarium* spp. by *Trichoderma* species is mostly studied on *F. oxysporum* and effectiveness of

Trichoderma spp. against *F. oxysporum* f. sp. *ubense* (Thangavelu et al., 2004), *F. oxysporum* f. sp. *adzuki* (John et al., 2010), *F. oxysporum* f. sp. *vasinfectum* (Zang et al., 1996), *F. oxysporum* f. sp. *lycopersici* (Cotxarrera et al., 2002), and *F. oxysporum* f. sp. *phaseoli* (Sallam et al., 2008) was proved *in vitro*. Maximal RGI in dual culture test with *F. oxysporum* f. sp. *phaseoli* was 51.4% (Sallam et al., 2008), while isolates tested herein were more effective considering that minimal RGI registered was 53.9%. Potential antagonism of *Trichoderma* spp. to *F. graminearum* is tested *in vitro* by Foroutan (2013), and mycelial growth of *F. graminearum* was inhibited from 28.5 – 58.8% which is much lower than in this study (60.5 - 71.1%). Additionally, effectiveness of *Trichoderma* species in contact growth inhibition of *F. verticillioides* was confirmed by Sempere & Santamarina (2009) and Calistruet al. (1997) as well as in this study.

Figure 1B indicates that there is no significant change in RGI scored on 14th and 21st day for *A. pinodella*, *F. proliferatum*, *F. verticillioides*, *F. oxysporum*, *P. teres*, and *S. sclerotiorum* while for other pathogens final RGI should be scored on 21st day.

According to Figure 1C, it can be noticed that *Trichoderma* spp. isolates covered colonies of tested phytopathogens surfaces until 14th day: completely (grade 1), up to 2/3 (grade 2) and half of surface (grade 3), while on 21st day grades 1 and 2 dominated which classed *Trichoderma* isolates as effective competitors. The exception was one *Trichoderma* isolate which was dominated by *S. sclerotiorum* (grade 4, Figure 1C). More than 50% of tested isolates completely covered *S. sclerotiorum* colonies (grade 1) but contact inhibition did not stopped pathogens mycelia growth which resulted in low RGI on 14th and 21st day (Figure 1B). De Figueirêdo et al. (2010) reported that 37.5% of tested *Trichoderma* spp. isolates expressed satisfactory antagonism against *S. sclerotiorum* (grade 1), while 62.5% expressed mild antagonism (grade 2). After all, in this study most *Trichoderma* spp. isolates expressed satisfactory antagonistic potential, especially against pathogens *M. phaseolina*, *A. pisi*, *A. pinodes*, *P. teres* and other than *S. sclerotiorum*.

Conclusions

Study confirmed that tested isolates of *Trichoderma* spp. are effective biocontrol agents against pathogens such as *M. phaseolina*, *P. teres*, *Ascochyta* complex and *Fusarium* spp. *in vitro*, and after additional tests and molecular identification, potentially can be used in biocontrol of those pathogens *in vivo*. These are first reports of *Trichoderma* spp. *in vitro* efficacy against *A. pisi*, *A. pinodes*, *A. pinodella*, and *P. teres*. After all, results of this study confirmed that *S. sclerotiorum* is still one of the most aggressive pathogens and remains as a big challenge for *Trichoderma* spp. and biocontrol.

References

- Ali, S. M., Nitschke, L. F., Dube, A. J., Krause, M. R., Cameron, B. (1978). Selection of pea lines for resistance to pathotypes of *Ascochyta pinodes*, *A. pisi* and *Phoma medicaginis* var. *pinodella*. *Crop and Pasture Science*, 29(4), 841-849.
- Ali-Haimoud, D. E., Marrault, M., Albertini, L. (1993). Evaluation of organisms antagonistic to the sclerotoid organs of *Drechslera teres*, the causal agent of barley net blotch. *Plant Disease*, 77, 1251-1255.
- Barilli, E., Cobos, M. J., Rubiales, D. (2016). Clarification on Host Range of *Didymella pinodes* the Causal Agent of Pea Ascochyta Blight. *Frontiers in Plant Science*, 7, 592.
- Benzohra, I. E., Bendahmane, B. S., Labdi, M., Benkada, Y. (2011). In vitro Biocontrol Using the Antagonist *Trichoderma harzianum* Against the Algerian Isolates of *Ascochyta rabiei* (Pass.) Labr., the Agent of Ascochyta Blight in Chickpea (*Cicer arietinum* L.). *International Journal of Microbiological Research*, 2 (2), 124-128.
- Bhatti, M. A., Craft, J. M., (1992). Influence of soil moisture on root rot and wilt of chickpea. *Plant Disease*, 76, 1259-1262.
- Bolton, D. M., Thomma, B. P. H. J., Nelson, B. D. (2006). *Sclerotinia sclerotiorum* (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. *Molecular Plant Pathology*, 7(1), 1-16.
- Bottalico, A. (1998). *Fusarium* Diseases of Cereals: Species Complex and Related Mycotoxin Profiles in Europe. *Journal of Plant Pathology*, 80 (2), 85-103.
- Bottalico, A., Perrone, G. (2002). Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. (A. Logrieco, J. A. Bailey, L. Corazza, B. M. Cooke eds.), *Mycotoxins in Plant Disease*. Kluwer Academic Publishers: 611-624.
- Calistru, C., McLean, M., Berjak, P. (1997). *In vitro* studies on the potential for biological control of *Aspergillus flavus* and *Fusarium moniliforme* by *Trichoderma* species. *Mycopathologia*, 139, 115-121.
- Cotxarrera, L., Trillas-Gay, M. I., Steinberg, C., Alabouvette, C. (2002). Use of sewage sludge compost and *Trichoderma asperellum* isolates to suppress *Fusarium* wilt of tomato. *Soil Biology and Biochemistry*, 34, 467-476.
- Da Silva, V. N., Fernandes, F. M., Cirtéz, A., Ribeiro, D. H., De Almeida, A. P., Hasegawa, R. H., Correa, B. (2006). Characterization and genetic variability of *Fusarium verticillioides* strains isolated from maize and sorghum in Brazil based on fumonisins production, microsatellites, mating type locus, and mating crosses. *Canadian Journal of Microbiology*, 52(8), 798-804.
- de Figueirêdo, G. S., de Figueirêdo, L. C., Cavalcanti, F. C. N., dos Santos, A. C., da Costa, A. F., de Oliveira, N. T. (2010). Biological and chemical control of *Sclerotinia sclerotiorum* using *Trichoderma* spp. and *Ulocladium atrum* and pathogenicity to bean plants. *Brazilian Archives of Biology and Technology*, 53 (1), 1-9.
- Doohan, F. M., Brennan, J., Cooke, B. M. (2003). Influence Of Climatic Factors on *Fusarium* Species Pathogenic to Cereals. *European Journal of Plant Pathology*, 109, 755-768.
- Dulić, K. (1987). Najčešće bolesti soje u Zemun Polju 1983. godine. *Arhiv poljoprivrednih nauka* 172,429-436.
- Elmer, W. H., Summerell, B. A., Burgess, L. W., Nigh Jr. E. L. (1999). Vegetative compatibility groups in *Fusarium proliferatum* from asparagus in Australia. *Mycologia*, 91 (4), 650-654.
- Foroutan, A. (2013). Evaluation of *Trichoderma* isolates for biological control of wheat *Fusarium* foot and root rot. *Romanian Agricultural Research*, 30, 335-342.
- Furlong, E.B., Soares, L. M. V., Lasca, C. C., Kohara, E. Y. (2005). Mycotoxins and Fungi in Wheat Harvested During 1990 in Test Plots in the State of São Paulo, Brazil. *Mycopathologia*, 131(3), 185-190.
- Howel, C. R. (2003). Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. *Plant disease*, 87, 4-10.
- Jayasena, K. W., van Burgel, A., Tanaka, K., Mejewski, J., Loughman, R. (2007). Yield reduction in barley in relation to spot-type net blotch. *Australasian Plant Pathology*, 36, 429-433.
- Jasnić, S. M., Vidić, M. B., Bagi, F. F., Đorđević, V. B. (2005). Pathogenicity of *Fusarium* Species In Soybean. *Proceedings for Natural Sciences Matica Srpska Novi Sad*, 109, 113-121.
- Jiménez-Díaz, R. M., Blanco-López, M. A., Sackston W. E. (1983). Incidence and distribution of charcoal rot of sunflower caused by *Macrophomina phaseolina* in Spain. *Plant Disease*, 63, 1033-1036.
- John, R. P., Tyagi, R. D., Prévost, D., Brar, S. K., Pouleur, S., Surampalli, R.Y. (2010). Mycoparasitic *Trichoderma viride* as a biocontrol agent against *Fusarium oxysporum* f. sp. *adzuki* and *Pythium arrhenomanes* and as growth promoter of soybean. *Crop Protection*, 29, 1452-1459.
- Joshi, B. B., Bhatt, R. P., Bahukhandi, D. (2010). Antagonistic and plant growth activity of *Trichoderma* isolates of Western Himalayas. *Journal of Environmental Biology*, 31(6), 921-928.
- Krnjaja, V., Lević, J., Tomić, Z., Stanković, S. (2007). Diversity of vegetative compatibility of *Fusarium oxysporum* isolated from alfalfa roots in Serbia. *Journal of Mountain Agriculture on the Balkans*, 10(5), 973-986.
- Leslie, J. F., Pearson, C. A. S., Nelson, P. E., Tousoun, T. A. (1990). *Fusarium* species from corn, sorghum and soybean fields in the Central and Eastern United States. *Phytopathology*, 80, 343-350.
- Leslie, J. F., Summerell, B. A. (2006). *The Fusarium Laboratory Manual*. Ames, IA, USA: Blackwell Publishing.
- Logrieco, A., Mule, G., Moretti, A., Bottalico, A. (2002). Toxigenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe. (A. Logrieco, J.A. Bailey, L. Corazza, B.M. Cooke, eds.), *Mycotoxins in Plant Disease*. Kluwer Academic Publishers: 597-609.
- Ma, Z., Lapitan, N.L. V., Steffenson, B.J. (2004). QTL mapping of net blotch resistance genes in a doubledhaploid population of six-row barley. *Euphytica*, 137(3), 291-296.
- Marić, A., Čamprag, D., Maširević, S. (1988). White rot (*Sclerotinia sclerotiorum*). In: (Milošević S. ed.) Diseases and pests of sunflower and their control. Nolit, Belgrade, 69-83.
- McDonald, G. K., Peck, D. (2009). Effects of crop rotation, residue retention and sowing time on the incidence and survival of ascochyta blight and its effect on grain yield of field peas (*Pisum sativum* L.). *Field Crops Research*, 111(1), 11-21.
- McLean, M. S., Howlett, B. J., Hollaway, G. J. (2009). Epidemiology and control of spot form of net blotch (*Pyrenophora teres* f. *maculata*) of barley: a review. *Crop Pasture Science* 60, 303-315.
- Menzies, J. G. (1993). A strain of *Trichoderma viride* pathogenic to germinating seedlings of cucumber, pepper and tomato. *Plant Pathology*, 42, 784-791.
- Monte, E. (2001). Understanding *Trichoderma*: Between biotechnology and microbial ecology. *International Microbiology*, 4, 1-4.
- Moretti, A. (2009). Taxonomy of *Fusarium* Genus, a Continuous Fight Between Lumpers and Splitters. *Proceedings for Natural Sciences Matica Srpska Novi Sad*, 117, 7-13.
- Nyvall, R. F., Percich, J. A., Mirocha, C. J. (1999): *Fusarium* head blight of cultivated and natural wild rice (*Zizania palustris*) in Minnesota caused by *Fusarium graminearum* and associated *Fusarium* spp. *Plant disease* 83, 159-164.
- Onyike, N. B. N., Nelson, P. E., Marasas, W. F. O. (1991): *Fusarium* species associated with millet grain from Nigeria, Lesatho, and Zimbabwe. *Mycologia* 83, 708-712.
- Peever, T. L., Barve, M. P., Stone, L. J., Kaiser, W. J. (2007). Evolutionary relationships among *Ascochyta* species infecting wild and cultivated hosts in the legume tribes *Cicereae* and *Vicieae*. *Mycologia*, 99(1), 59-77.
- Sallam, N. M. A., Abo-Elyousr, K. A. M., Hassan, M. A. E. (2008). Evaluation of *Trichoderma* species as biocontrol agents for damping-off and wilt diseases of *Phaseolus vulgaris* L. and efficacy of suggested formula. *Egyptian Journal of Phytopathology*, 36(1-2), 81-93.
- Sempere, F., Santamarina, M. P. (2009). Antagonistic interactions between fungal rice pathogen *Fusarium verticillioides* (Sacc.) Nirenberg and *Trichoderma harzianum* Rifai. *Annals of Microbiology*, 59(2), 259-266.
- Singh, V., Ranaware, A. M., Nimbkar, N. (2008). Bioefficacy of antagonists against root-rot fungus *Macrophomina phaseolina* of safflower. 7th International Safflower Conference, 3-6th November 2008, Waga Waga, Australia.
- Stanković, S., Lević, J., Petrović, T., Krnjaja, V. (2008). Mycotoxin production by *Fusarium proliferatum* and *F. verticillioides* isolated from hops in Serbia. *Journal of Plant Pathology*, 90 (3), 58.

- Steffenson, B. J., Webster, R. K. (1992). Pathotype diversity of *Pyrenophora teres* f. *teres* on barley. *Phytopathology*, 82, 170-177.
- Tančić, S., Dedić, B., Dimitrijević, A., Terzić, S., Jocić, S. (2012). Bioecological relations of sunflower pathogens – *Macrophomina phaseolina* and *Fusarium* spp. and sunflower tolerance to these pathogens. *Romanian Agricultural Research*, 29, 349-359.
- Tančić, S., Stanković, S., Lević, J. (2009). Varijabilnost patogenih svojstava *Fusarium* spp. poreklom iz zrna pšenice i kukuruza. *Pesticides & Phytomedicine*, 24(4), 259-269.
- Thangavelu, R., Palaniswami, A., Velazhahan, R. (2004). Mass production of *Trichoderma harzianum* for managing fusarium wilt of banana. *Agriculture, Ecosystems & Environment*, 103, 259-263.
- Tomczak, M., Wiśniewska, H., Stepień, Ł., Kostecki, M., Chelkowski, J., Goliński, P. (2002). Deoxynivalenol, Nivalenol and Moniliformin in Wheat Samples with Head Blight (Scab) Symptoms in Poland (1998-2000). In: *Mycotoxins in Plant Disease* (A. Logrieco, J. A. Bailey, L. Corazza, B.M. Cooke eds). European Journal of Plant Pathology, 108, 625 – 630. Kluwer Academic Publishers.
- Varga, J., Tóth, B., Mesterházy, Á., Téren, J., Fazekas, B. (2002). Mycotoxigenic fungi and mycotoxins in foods and feeds in Hungary. In: *An Overview on Toxigenic Fungi and Mycotoxins in Europe* (A. Logrieco i A. Visconti eds). Kluwer Academic Publishers, London, 123 – 139.
- Vinale, F., Sivasithamparam, K., Ghisalberti, E., Marra, R., Woo, S. L., Lorito, M. (2008). *Trichoderma* – plant – pathogen interactions. *Soil Biology & Biochemistry*, 40, 1-10.
- Wallen, V. R. (1974). Influence of three ascochyta diseases of peas on plant development and yield. *Canadian Plant Disease Survey*, 54, 86-90.
- Zang, J., Howell, C. R., Starr, J. L. (1996). Suppression of *Fusarium* colonization of cotton roots and *Fusarium* wilt by seed treatments with *Gliocladium virens* and *Bacillus subtilis*. *Biocontrol Sciences and Technology*, 6, 175-187.
- http://eol.org/pages/187980/hierarchy_entries/57331216/overview [retrieval date: 06.03.2017.]
- <https://projects.ncsu.edu/cals/course/pp728/Macrophomina/host.htm> [retrieval date: 06.03.2017.]

Efikasnost *Trichoderma* spp. izolata protiv najčešćih gljivičnih patogena

Sonja Tančić Živanov · Radivoje Jevtić · Mirjana Lalošević ·
Dalibor Živanov · Slađana Medić-Pap · Vesna Župunski

Sažetak: U današnje vreme, organska proizvodnja sve više zahteva primenu ekoloških metoda u suzbijanju biljnih bolesti. Biološka kontrola bazirana na upotrebi *Trichoderma* vrsta obećava jer su *Trichoderma* vrste izuzetno agresivne prema velikom broju fitopatogenih gljiva. Stoga je cilj ovih istraživanja bio da se *in vitro* testira antagonističko dejstvo deset *Trichoderma* spp. izolata poreklom iz Srbije na deset ekonomski značajnih fitopatogenih gljiva. Istraživanja su potvrdila da su testirani izolati *Trichoderma* spp. inhibirali radijalni porast vrsta do: *Ascochyta pinodella* (76,9%), *A. pinodes* (60,0%), *A. pisi* (68,5%), *Fusarium graminearum* (71,1%), *F. proliferatum* (63,9%), *F. verticillioides* (62,6%), *F. oxysporum* (63,9%), *Macrophomina phaseolina* (63,8%) i *Pyrenophora teres* (83,9%). Najniža inhibicija porasta patogena je registrovana u dvojnim kulturama sa vrstom *S. sclerotiorum* – do 52,2% inhibicije.

Ključne reči: *Ascochyta* spp., *Fusarium* spp., *M. phaseolina*, *P. teres*, *S. sclerotiorum*, *Trichoderma* spp.

Received: 15 June 2017, Accepted: 10 October 2017

Published online: 4 December 2017

