EFFICACY OF *TRICHODERMA* SPP. ISOLATES AGAINST *SCLEROTINIA SCLEROTIORUM* ON SUNFLOWER SEEDLINGS

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ABSTRACT

Trichoderma species are well known as effective antagonists to a variety of soil fungal pathogens. The aim of this research was to test the ability of *Trichoderma* spp. isolates, which previously indicated antagonistic activity (Tančić et al. 2012), to protect sunflower seedlings from *Sclerotinia sclerotiorum*. Ten *Trichoderma* spp. isolates obtained from different soil types and localities in Vojvodina province and one *S. sclerotiorum* isolate from sunflower grown at Rimski Šančevi (Serbia) were used in research. Biological efficacy was tested on 100 sunflower seeds treated with *Trichoderma* spp. suspensions ($1x10^6$) in two different treatments: T-30 (modified Mukhtar et al., 2012) and T-1.2 (Maslienko, 2005). *Trichoderma*-coated seeds were placed in four replicates on wet filter paper in Petri dishes. Next to each *Trichoderma*-coated seed the 5 mm^2 plug of *S. sclerotiorum* mycelia was placed, and incubated under the optimal laboratory conditions. Seeds treated with sterile distilled water with pathogen and without it were used as a positive and negative control, respectively. After seven days, biological efficacy of *Trichoderma* spp. isolates was assessed and calculated according to Liu et al. (2009). According to obtained results, biological efficacy of all tested *Trichoderma* isolates was statistically significant as compared to the positive control in both treatments. Good antagonism with over 50% of biological efficacy was registered in 8 isolates in T-30, and 3 isolates in treatment T-1.2. Three *Trichoderma* isolates which showed biological efficacy over 50% in both treatments can be considered as potential biocontrol agents which should be included in further more comprehensive research.

Key words: *Trichoderma*, *Sclerotinia sclerotiorum*, Antagonism, Sunflower

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important annual species grown in Serbia mostly for its edible oil. Areas grown under sunflower were around 170 000 hectares with the expected yield of 422 000 tones and sunflower oil production of 139 000 tones in economical year 2015/2016 (Chamber of Commerce and Industry of Serbia, 2016).

Farmers around the world are familiar with *S. sclerotiorum* (Lib.) de Bary as a threat to numerous crops such as sunflower, soybean, oilseed rape, edible dry bean, chickpea, peanut, dry pea, lentils and various vegetables as well. Occurrence of diseases caused by *S. sclerotiorum* on sunflower is influenced by genotype and weather conditions. Moist and cold weather conditions prevailing in temperate climate regions favours *S. sclerotiorum* development. In Serbia, weather conditions favoured economically important Sclerotinia development on sunflower head in 1999 and 2005 with diseased plants even over 60% (Maširević and Forgić, 2000; Maširević and Dedić, 2006). Diseases caused by this fungus can

appear during the whole sunflower growing season, and yield loss depends on the sunflower development stage in which the disease occurs. Sunflower plants infected at the beginning of flowering stage can lose up to 98% of their potential yield, while plants infected eight weeks after flowering can lose not more than 12% of their potential yield (Maširević & Gulya 1992). The major control method for *Sclerotinia* diseases has been fungicide application in combination with host resistance.

Considering growing demand for organic food production, biocontrol of such cosmopolitan and devastating pathogen is a big challenge. The use of biofertilizers and biopesticides is an alternative for sustaining high eco-friendly production. Integrated control is facilitated by the fact that *Trichoderma* species are resistant to most chemical pesticides (Harman, 2011; FRAC, 2016). *Trichoderma* species have been known since 1930s (Weindling, 1932), but since 1990s their usage in commercial agriculture has been increased (Harman 2004, 2011). So far, *Trichoderma* species have been known as effective on nutrient utilization with high reproductive potential which allow them to survive under unfavourable conditions and makes them very competitive. Presence of great variety of lytic enzymes (cell-wall degrading enzymes) and secondary metabolites (gliotoxin, gliovirin, viridin, viridiol etc.) makes *Trichoderma* strongly aggressive to broad range of phytopathogenic fungi (Vinale et al., 2008). The main biocontrol mechanisms of *Trichoderma* species, when direct confrontation with pathogen occurs, are mycoparasitism and antibiosis (Howel, 2003). Another mechanism which is quite effective as well, but do not consider direct confrontation with the pathogen, is competition for soil nutrients and space. Additionally, *Trichoderma* species are known as well as plant growth promoter agents and promoters of plant defense mechanisms (Shoresh et al., 2010; Harman, 2011). *Trichoderma* colonizes roots and provides at least season-long benefits to plants, although it can be even for life because the best strains fully colonize roots as they grow (Harman 2000). So far it is recorded that *Trichoderma* species improve growth of lettuce, tomato, pepper, wheat, maize, soybean, chilli (Vinale et al., 2004; Tucci et al., 2011; Sukla et al., 2015; Maisuria and Patel, 2009; Asaduzzaman et al., 2010). Also, *Trichoderma* spp. is stimulating defense responses in its host plants and is known as one of the best induced systemic resistance (ISR) agents (Shoresh et al., 2010; Shoresh, 2005).

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, one should bear in mind 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 ability of native Serbian *Trichoderma* strains to protect sunflower seedlings in early stage from pathogen *S. sclerotiorum*.

MATERIAL AND METHODS

Plant and fungal material used: Fungal material was obtained from soil samples originated from different soil types and localities in Serbia, mainly from Vojvodina province. All *Trichoderma* spp. isolates were refined to single-spore according to Leslie and Summerell (2006). Ten isolates which previously indicated good antagonistic activity in dual culture test (Tančić et al., 2012) were selected for this research. Pathogen *S. sclerotiorum* was isolated from diseased sunflower plant at Rimski Šančevi.

Trichoderma isolates' efficacy against *S. sclerotiorum* was tested on sunflower seeds of sterile parental line VL-A-8A.

Preparation of conidia suspension: A conidia suspensions of ten tested *Trichoderma* isolates were prepared from 7-days old isolates by flooding method. Such suspensions were filtered through cheesecloth, and conidial concentrations were adjusted to 10^6 conidia/ml by Neubauer's haemocytometer. Additionally, suspensions were amended according to Mukhtar et al. (2012) method.

Treatment T-1.2 considered that seeds were treated with 1.2 µl of *Trichoderma* suspension which was equally distributed per g of seeds and air dried on filter paper in Petri plates for 24 hours at room temperature (Маслиенко, 2005). Control was treated with 1.2 µl of sterile distilled water per g of seeds.

Treatment T-30 considered that seeds were dipped in seed-coating suspensions for 30 minutes and air dried on filter paper in Petri plates for 24 hours at room temperature, while sterile distilled water was used as a control.

Biological efficacy test: was done on 100 sunflower seeds treated with *Trichoderma* suspensions of different intensities (T-1.2 and T-30). Treated seeds were germinated in four replicates on double wet filter paper. Next to each sunflower seed, the 5 mm^2 plug of potato dextrose agar (PDA) with 7-day old micelia of *S. sclerotiorum,* was placed. Seeds treated with sterile distilled water without presence of pathogen *S. sclerotiorum* plugs were used as negative control, while seeds treated with sterile distilled water with presence of *S. sclerotiorum* plugs were used as positive control. Seeds were germinated in growth chamber with 12h photoperiod at 25±1ºC. After seven days diseased seedlings and seeds were counted, and biological efficacy of the *Trichoderma* isolate was calculated according to formula (Liu et al. 2009):

$$C\ (\%) = 100 * (a - b) / a$$

where C is biological efficacy in %, a – number of diseased seeds and seedlings in positive control, and b – number of diseased seeds and seedlings in treatment.

Beside biological efficacy, germination (G) was calculated as well on 7th day of the experiment.

Statistical analyses: All obtained data were analyzed in Statistica 12 using Duncan's test (percentages were previously transformed in ArcSin√%).

RESULTS AND DISCUSSION

Formation of rhizosphere microflora occurs usually in first three days after germination, and its progress in the deeper soil layers follows root growing and stimulates plant exometabolites at the same time (Асатурова, 2009). This is very important in biocontrol especially because young seedlings are often infected by pathogens in early stage of their development. Due to above mentioned, biological efficacy was estimated in the first days of sunflower germination and expressed as a percentage of protected seeds and seedlings comparing positive control (seeds without *Trichoderma* treatment grown in presence of pathogen *S. sclerotiorum*).

Germination was calculated on 7th day of incubation. Lower germination rates were registered in treatments with lower biological efficacy. Biological efficacy of all tested isolates was statistically significant as compared to the positive control in both treatments. According to obtained results, biological efficacy of tested *Trichoderma* isolates varied from 36 – 68% and 23.8 – 60.6% for treatments T-30 and T-1.2 respectively (Table 1). Excellent

antagonism with over 50% of biological efficacy was registered in 8 isolates in T-30, and 3 isolates in treatment T-1.2 (bold values in Table 1). Three *Trichoderma* isolates – K150, K173 and K174 showed biological efficacy over 50% in both treatments. These are promising results considering that some authors with bacterial antagonist reached biological efficacy against *Fusarium* spp. on sunflower seedlings from 0-36% (Асатурова, 2009), while biological efficacy of fungal and bacterial antagonist against *S. sclerotiorum* on sunflower stem under the field conditions was much higher - 54.5-100% (Фирсов et al., 2009). Besides on sunflower, the antagonistic activity of *Trichoderma* spp. against *S. sclerotiorum* was proven on other crops as well. Thus, the application of *T. harzianum* as alignate capsules increased the survival of soybean plants more than 100% and 40% in greenhouse and in the field, respectively (Menendez and Godeas, 1998). Isolates of *T. harzianum* also protected over 80% of tomato, squash and eggplant seedlings inoculated with *S. sclerotiorum* in the greenhouse experiments (Abdullah et al., 2008). Further, *T. virens* significantly reduces the percentage of viable sclerotia and number of apothecia produced (Huang and Erickson, 2008) which can be used for bioregulation of pathogen density in soil.

Table 1. Biological efficacy of two different treatments with *Trichoderma* spp. isolates against *S. sclerotiorum* on sunflower seedlings

	T-30		T-1.2	
Isolate No	C (%)	G (%)	C (%)	G (%)
K114	**50.0^{ab}**	86^{ab}	26.2^{bc}	78^{a}
K132	36.0^{b}	74^{a}	45.2^{a}	84^{ab}
K150	**58.0^{ac}**	94^{ab}	**58.3^{a}**	80^{ab}
K160	**50.0^{ab}**	90^{ab}	40.5^{abc}	77^{a}
K173	**56.0^{ac}**	76^{a}	**54.8^{a}**	85^{ab}
K174	**58.0^{ac}**	86^{ab}	**60.6^{a}**	85^{ab}
K175	**68.0^{c}**	74^{a}	23.8^{b}	91^{b}
K176	**59.5^{a}**	88^{ab}	48.0^{ab}	87^{ab}
K178	40.0^{b}	90^{ab}	42.9^{ac}	80^{ab}
K179	**53.6^{a}**	98^{b}	44.0^{ab}	87^{ab}
- Control	100^{d}	88^{ab}	100^{d}	96^{ab}
+ Control	0.00^{e}	80^{ab}	0.00^{e}	82^{ab}

Legend: Values in the columns followed by the same letters are not significantly different ($p<0.05$) by Duncan's test; Values are average of four replicates;

Beside *S. sclerotiorum*, it has been proven that *Trichoderma* spp. are aggressive to broad range of phytopathogenic fungi – *Rhizoctonia, Fusarium, Alternaria, Ustilago, Venturia, Colletotrichum, Pythium, Phytophthora, Thielaviopsis, Sclerotium cepivorum, Sclerotinia minor* etc. (Vinale et al., 2008; Thomas et al., 2004; McLean et al., 2012).

All mentioned above is leading to a conclusion that those perspective isolates from our research could also be good antagonists for some other important sunflower pathogens which should be tested in some further research.

CONCLUSION

Three out of ten tested *Trichoderma* isolates originating from Serbia expressed excellent ability to protect sunflower seedlings from pathogen *S. sclerotiorum* in both treatments. Those isolates can be considered as potential biocontrol agents and should be included in further, more comprehensive, research.

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