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Scientific paper

Impact of *Trichoderma* spp. on Soybean Seed Germination and Potential Antagonistic Effect on *Sclerotinia sclerotiorum*

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SUMMARY

Trichoderma species have been registered as species with important plant growth promoting potential and antagonistic effect against various phytopathogens. *Trichoderma* isolates originating from different soil types from the Vojvodina region (Serbia) were screened using dual culture test for their antagonistic effect against the pathogen *Sclerotinia sclerotiorum*. All tested isolates had high radial growth inhibition (RGI) factors of the pathogen and high colonization percentage. Growth promoting activity of *Trichoderma* isolates on soybean seeds was tested under glasshouse conditions. Soybean seeds were coated with suspensions of different *Trichoderma* isolates and seed germination percentage, root and shoot length were measured. According to data analysed in Statistica 10, using Duncan's test, there were no significant effects on shoot length among the tested isolates, compared to the control. The four tested *Trichoderma* isolates showed significant positive effects on germination, root length and vigour index, while two isolates exhibited no significant effect on any of the measured parameters.

Keywords: *Trichoderma*; Seeds; Soybean; Germination

INTRODUCTION

Biocontrol and other environment friendly pest management practices have become one of the driving forces in recent development of organic agriculture. Selection of biocontrol agents with wide host ranges is therefore one of the main goals for commercial manufacturing of biopesticides. Nowadays, numerous commercial biofungicides, such as Bio-Fungus (Belgium), Trichodex (Israel), Trieco (India), Supresivit (U.S., Europe), Promot (California), Binab T (U.K., Sweden), Root Shield (USA, Europe), etc., are based on *Trichoderma viride*, *T. harzianum* and *T. koningii* strains that are efficient biocontrol agents of several phytopathogenic fungi (https://attra.ncat.org/attra-pub/summaries/summary.php?pub=146). So far, *Trichoderma* species have been mainly registered as effective antagonists of soilborne pathogens, such as *Sclerotinia sclerotiorum* (Mansour et al., 2008; Matroudi et al., 2009; Ibarra-Mediná et al., 2010), *S. minor* (Jones and Stewart, 1997; Ibarra-Mediná et al., 2010), *Macrophomina phaseolina* (Ilyas et al., 1985; Larralde-Corona et al., 2008), *Rhizoctonia solani* (Maisura and Patel, 2009; da Silva et al., 2012), *Fusarium* spp. (Sempere and Santamarina, 2009; Zang and Wang, 2012), *Pythium* spp. (Maisura and Patel, 2009; Mishra, 2010), *Phytophtora* spp. (Mpika et al., 2009), etc. In addition to their antagonistic activity, *Trichoderma* strains have also demonstrated growth promoting properties – enhancing germination, shoot and root length and vigour indexes of various plant seeds. Seed treatments can be used on many crops for various purposes but mainly to improve plant growth and productivity of seeds.

As it was mentioned above, *Trichoderma* species are effective antagonists of the cosmopolite pathogen *Sclerotinia sclerotiorum*, which is found worldwide in many different soil types and environmental conditions and causes diseases of more than 400 plant species. This pathogen is common on various vegetable plants, sunflower, soybean, edible dry bean, chickpea, peanut, etc., causing white rot of plant roots, stems and fruits or pods, which ultimately ends in a complete plant wilting or rotting if infection had started early. In the case of early infection, the percentage of yield reduction is almost equal to the percentage of infected plants. In years with high amounts of rainfall during summer, white rot is the most dangerous soybean disease in Serbia and its infection intensity can exceed 50%, causing drastic reduction in soybean yields (Vidić and Jasnić, 2011).

Trichoderma species have attracted considerable scientific attention in the past few decades as potential fungal biocontrol agents against a vast number of plant pathogens, and are attractive in a wider research field. The aim of the present study was to assess the growth promoting activities of *Trichoderma* isolates on soybean seeds and antagonistic activities of those isolates against the common pathogen *S. sclerotiorum*.

MATERIAL AND METHODS

The tested *Trichoderma* isolates originated from different soil types and localities in Vojvodina (Serbia), while the *S. sclerotiorum* isolate originated from a sunflower crop grown at Rimski Šančevi (Novi Sad, Serbia). The isolates were refined to monospores for further research.

Dual culture test was used for screening nine *Trichoderma* isolates and their antagonistic effects against *S. sclerotiorum in vitro*. Each *Trichoderma* isolate plug of a 7-day old culture (5 mm^2) was confronted with identical plugs of *S. sclerotiorum* isolates in 90 mm Petri plates at 60 mm distance on PDA in four replicates. The antagonistic abilities of *Trichoderma* isolates were evaluated periodically, i.e. on the 7^{th}, 14^{th}, 21st and 28^{th} day of incubation in the dark at 25°C. After 28 days of incubation, Radial Growth Inhibition (RGI) and Colonization percentages (C) were calculated according to Rodriguez et al. (2000) and Ibarra-Medina et al. (2010), respectively.

The growth promoting activity of the tested *Trichoderma* isolates on soybean seeds (Favorit variety) were tested under glasshouse conditions. One hundred of surface sterilized soybean seeds were coated with suspensions of each *Trichoderma* isolate according to a modified method proposed by Mukhtar et al. (2012), while sterile distilled water was used as a control. Spore suspension concentrations were adjusted to $1x10^6$ conidia/ml by haemocytometer. Previously sterilized soybean seeds were dipped in seed coating suspensions for 30 minutes and then air dried on filter paper in Petri plates for 24 hours. Dry *Trichoderma* coated seeds were sown in four replications (25 seeds per pot sized 12.5 x 17 cm) in Classman substrate 2. Seed germination percentages and root and shoot lengths were measured 7 days after sawing. Vigour index was calculated according to formula (Asaduzzaman et al., 2010):

Vigour index = [Mean of root length (cm) + Mean of shoot length (cm)] x percentages of seed germination.

All obtained data were analyzed in Statistica 10 using Duncan's test.

RESULTS AND DISCUSSION

According to the results obtained in dual culture tests *in vitro*, the best inhibition of *S. sclerotiorum* radial growth (RGI) was observed in interaction with the *Trichoderma* isolate K132 (52.2%), while *Trichoderma* isolate K174 achieved the best colonisation of the pathogen (100%).

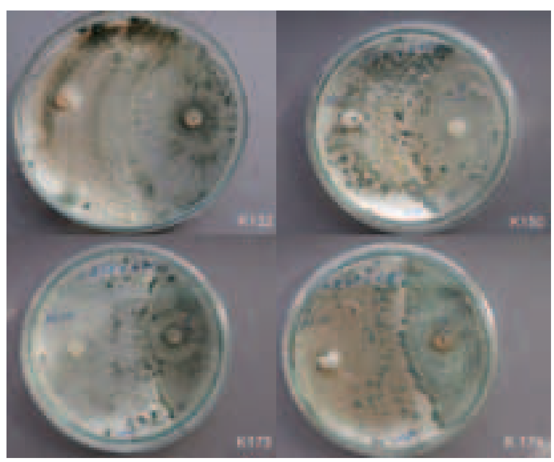

Figure 1. *Trichoderma* isolates with the highest colonization percentages and RGI factors in dual culture tests with *S. sclerotiorum*

What is more, all tested *Trichoderma* isolates except K175 caused high colonisation of pathogen mycelia of more than 70% (Table 1). According to Ibarra-Medina et al. (2010), isolates with over 70% colonized pathogen mycelia can be considered as effective antagonists. Considering this research result, four isolates (K173, K174, K176 and K178) can be defined as highly invasive, with colonisation percentages exceeding 90% (Table 1), while Ibarra-Medina et al. (2010) found only 10 out of 71 tested *Trichoderma* isolates to be highly invasive for *S. sclerotiorum* and *S. minor* with colonisation percentages of over 90%. Matroudi et al. (2009) reported RGIs as high as 93% in dual culture tests with *T. atroviride* and *S. sclerotiorum* isolates, while the *Trichoderma* isolates tested in our research had much lower inhibitory effects on the pathogen's mycelial growth. *Trichoderma* isolates originating in the Western Himalayas had been found to significantly inhibit *S. sclerotiorum* mycelial growth, from 53.27% up to 82.73%. Among all tested Himalayan *Trichoderma* isolates, 10% were highly inhibitory to *S. sclerotiorum* mycelial growth with RGIs of over 80%, while 53% caused inhibition with RGIs between 60-80%, 35% caused inhibition with RGIs between 45-60%, and 2% with RGI less than 45% (Joshi et al., 2010).

Similar data showing *Trichoderma* isolates' antagonistic effect on other pathogen species have also been reported. Antagonism of *Trichoderma* isolates in dual culture tests was recorded on *Pythium aphanidermatum* by Mishra (2010) and maximal inhibition of pathogen micelial growth (RGI) was 72% in a dual culture test with a *Trichoderma viride* isolate. Nine *Trichoderma* spp. isolates originating from Mexican sandy soils have been found to reduce radial growth rate of *Macrophomina phaseolina* isolates by at least 50% using *in vitro* dual culture test (Larralde-Corona et al., 2008), while RGI of *M. phaseolina* mycelia by *Trichoderma harzianum* was 47% (Ilyas et al., 1985). *Trichoderma* isolates have also inhibited radial growth of *Phytophtora palmivora* mycelia by 33.29-97.86% (Mpica et al., 2009).

In glasshouse tests, there were no significant effects of any of the tested *Trichoderma* isolates on shoot length, compared to the control. In contrast, significant positive effects on both root length and germination were found in treatments with four *Trichoderma* isolates – K114, K132, K150 and K160. Vigour indexes of plants treated with those four *Trichoderma* isolates were significantly higher than the control as well (Table 1). Some tested *Trichoderma* isolates exhibited significant positive effect only on root length (K176) or germination (K178), or germination and vigour index (K175), while two isolates (K173, K174) showed no significant effect on any measured parameter, despite their high colonisation ability against the pathogen in dual culture test (Table 1). In their research of the influence of rhizosphere fungi on soybean, Maisura and Patel (2009) found that *Trichoderma viride* promoted an increase in root length, shoot length and seed germination (96.6%). Also, Mukhtar et al. (2012) reported enhanced germination of soybean seeds treated with 6 different *Trichoderma* species, with germination varying from 76-96%, depending on species, which was higher than the germination obtained in the control (76%). In our research, all tested

Table 1. *Trichoderma* isolates' growth promoting effect (root length, germination and vigour index) on soybean seeds and antagonistic effect against *S. sclerotiorum in vitro*

Isolate Code	RGI (%)	C (%)	Germination percentage	Germination* (ArcSin %)	Root length* (mm)	Vigour Index*
K 114	39.5	76.4	75	59.43 ab	166.53 a	2023.82 ab
K132	52.2	80.0	74	59.43 ab	157.95 a	1893.81 ab
K150	43.9	84.2	79	62.97 a	166.61 a	2214.22 b
K160	41.7	87.5	75	61.63 ab	156.04 a	1979.09 ab
K173	36.1	95.8	73	58.74 abc	143.42 ab	1753.48 abc
K174	37.2	100.0	72	55.57 bc	134.44 ab	1684.52 ac
K175	40.5	46.7	78	62.15 a	147.54 ab	1877.99 ab
K176	37.2	92.5	68	58.19 abc	150.89 a	1825.21 abc
K178	40.0	90.0	75	60.12 ab	130.64 ab	1769.01 abc
Control			64	53.15 c	107.19 b	1357.65 c

*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

Trichoderma isolates also enhanced germination of soybean seeds, compared to the control. Lower germination percentages of the tested soybean seeds than usual were due to extremely high temperatures which occurred in Serbia during the grain filling stage in 2012 (http://www.hidmet.gov.rs/podaci/agro/godina.pdf). That is why this seed material was good for testing the growth promoting activities of *Trichoderma* isolates.

Trichoderma isolates were also found to have similar growth promoting effect on other grown plants, such as sunflower, rice, maize, bean, safflower, papper, chili, radish, cucumber, tomato, mustard etc. Ilyas et al. (1985) reported that *T. harzianum* isolates significantly increased plant height (16.2%) and dry stem weight (10.9%), compared to non-treated healthy sunflower plants in a pot experiment. The growth promoting effects of *T. viride* and *T. harzianum* isolates on safflower were tested in a pot experiment, and germination percentages (91.1% and 86.7% respectively), radicale and plumule lenghts were significantly higher than those of untreated seeds in a control (Singh et al., 2008). Joshi et al. (2010) found the maximal germination percent in chilli treated with *Trichoderma* isolates (79.49%) to be significantly higher than in a control, and enchanced root and shooth lenghts as well. To summarize, *Trichoderma* isolates tested in this research were equally effective as a growth promoting factor for soybean seeds as the *Trichoderma* strains tested so far on different other crops.

CONCLUSIONS

This research shows that eight out of nine *Trichoderma* isolates tested *in vitro* can be considered as effective antagonists against *S. sclerotiorum* based on their colonisation percentages that exceeded 70%. Also, four of those *Trichoderma* isolates had significant positive effects on germination, root length and vigour index of soybean seeds in the glasshouse experiment. Those isolates are promising antagonists and will be included in more comprehensive future research of their antagonistic effects against *S. sclerotiorum*.

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Uticaj *Trichoderma* spp. na klijavost semena soje i potencijalni antagonistički efekat na *Sclerotinia sclerotiorum*

REZIME

Vrste roda *Trichoderma* su poznate po svom antagonističkom efektu na veliki broj fitopatogenih organizama kao i po stimulativnom efektu za rast biljke. Izolati *Trichoderma* spp. testirani u ovom istraživanju su izolovani iz različitih tipova zemljišta u Vojvodini (Srbija). Test dvojnih kultura je korišćen za testiranje antagonističkog dejstva izolata na patogena *Sclerotinia sclerotiorum*. Svi testirani izolati su imali visok RGI (faktor inhibicije porasta micelije patogena) i visok procenat kolonizacije patogena. Stimulativni efekat rasta *Trichoderma* izolata na seme soje je testiran u uslovima staklenika, i sedmog dana su mereni dužina korena, izdanka i klijavost semena tretiranih suspenzijom različitih izolata *Trichoderma* spp. Svi podaci su analizirani primenom Duncan testa u programu Statistica 10, i ukazuju da nije bilo statistički značajne razlike u dužini izdanaka tretiranih semena i kontrole. Četiri izolata su statistički značajno uticala na povećanje dužine korena, klijavosti i vigor indeksa u odnosu na kontrolu, dok dva izolata nisu statistički značajno uticala ni na jedan od merenih parametara.

Ključne reči: *Trichoderma*; seme; soja; klijanje