Journal of Plant Protection Research ISSN 1427-4345

ORIGINAL ARTICLE

Application of organic waste material overgrown with *Trichoderma atroviride* as a control strategy for *Sclerotinia sclerotiorum* and *Chalara thielavioides* in soil

Beata Kowalska*, Urszula Smolińska, Magdalena Szczech, Jolanta Winciorek

Department of Microbiology, Research Institute of Horticulture, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland

Vol. 57, No. 3: 205–211, 2017

DOI: 10.1515/jppr-2017-0027

Received: February 10, 2017
Accepted: July 12, 2017

*Corresponding address:
Beata.Kowalska@inhort.pl

Abstract

The effect of granulated organic waste material overgrown with *Trichoderma atroviride* TRS25 on the survival of *Sclerotinia sclerotiorum* and *Chalara thielavioides* in the soil was investigated. Application of this material into the soil at a dosage of 1% (w/v) reduced the survival of *S. sclerotiorum* sclerotia to almost zero after 2 months of incubation. The sclerotia were parasitized by *T. atroviride* fungus multiplied on granulates. The detrimental effect of granulates on *Ch. thielavioides* was observed after 4 months of incubation. The granulates, with *Trichoderma* and without the fungus, caused a decrease of the pathogen population in soil. *Trichoderma atroviride* introduced into the soil as a conidia suspension did not decrease the amount of *Ch. thielavioides* but the fungus parasitized *S. sclerotiorum* sclerotia. After the addition of granulated waste material, an increase of bacteria, especially the *Pseudomonas* group in the soil was observed.

Key words: biocontrol, fruit pomaces, *Chalara thielavioides*, *Sclerotinia sclerotiorum*, *Trichoderma atroviride*

Introduction

The application of intensive methods of plant cultivation leads to the accumulation of harmful microorganisms and resting propagules of pathogens in the soil, which affects economically important plant species. The fungi *Sclerotinia sclerotiorum* (Lib.) de Bary and *Chalara thielavioides* (Peyr.) Nay Raj & Kendrick (Weber and Tribe 2004) belong to soilborne pathogens which produce long-lived survival forms, sclerotia and chlamydospores, which are difficult to eradicate and restrict the productivity of horticultural crops.

Sclerotinia sclerotiorum is the causative agent of white mold on more than 400 plant species. The disease is one of the main causes of yield losses of many vegetables, e.g. carrot, lettuce, parsley and bean (Kora *et al.* 2005; Johnson and Atallah 2014). On diseased plants the pathogen forms white, fluffy mycelium. Later, after several days black, irregular sclerotia are produced.

The fungus *Ch. thielavioides* is a common and important soil pathogen that causes damping of seedlings and black root rot of many crops (Agrios 2005). Infected plants develop black root rot and become stunted, chlorotic, and produce reduced yields of low quality. Black root rot is due to dark-colored chlamydospores produced by the fungus on the infected roots (Kowalska and Smolińska 2003). The disease is especially dangerous during carrot storage. The symptoms typically develop after the carrots have been washed, packaged in plastic and stored. The tissue wounds caused by postharvest brushing of carrots increased disease incidence, whereas avoiding the brush process often eliminated the development of black root rot disease during storage and post-storage shelf-life (Paulin-Mahady *et al.* 2002; Weber and Tribe 2004).

Currently the chemical or biological methods which are being used for the eradication of *S. sclerotiorum* and *Ch. thielavioides* from soil are not effective enough. One of the most effective ways to decrease the amount of *S. sclerotiorum* sclerotia in soil is the degradation

of sclerotia by fungal antagonists. Intense studies have been conducted on the parasitic fungus *Coniothyrium minitans* (McQuilken *et al.* 1995; Zeng *et al.* 2012). Contans®WG, a commercial formulation of *C. minitans* (strain CON/M/91-08), is known for its ability to reduce damage caused by *S. sclerotiorum* in several crops by infecting and degrading sclerotia in the soil (McQuilken and Chalton 2009). Among many reports concerning the activity of antagonists against *S. sclerotiorum*, the genus *Trichoderma* has also been extensively studied in the biocontrol of this pathogen. The potential control of soilborne diseases with *Trichoderma* spp. has raised considerable research interest in recent years (Matroudi *et al.* 2009; Druzhinina *et al.* 2011; Hermosa *et al.* 2012; Geraldine *et al.* 2013; Aleandri *et al.* 2015; Shafique *et al.* 2016). Searching for new control methods is important. One possible method could be using antagonistic microorganisms multiplied on organic carriers (Köhl *et al.* 2011; Smolińska *et al.* 2014a; Smolińska *et al.* 2014b; Smolińska *et al.* 2016). The following species: *T. virens*, *T. atroviride* and *T. harzianum* introduced into the soil on organic carriers significantly reduced the *S. sclerotiorum* population (Smolińska *et al.* 2016). In this antagonistic fungus mycoparasitism is the main mechanism of biocontrol activity. The effective production of hydrolytic enzymes degradating pathogen cell walls e.g. chitinase, glucanase, N-acetylglucosaminidase and protease is a key step in the successful establishment of a mycoparasitic relationship (Geraldine *et al.* 2013; Monfil and Casas-Flores 2014; Khaledi and Taheri 2016).

In the literature there are not any reports about the influence of *Trichoderma* spp. on the *Ch. thielavioides* population in a soil environment. However, biological methods for control of the pathogen are being considered (Eshel *et al.* 2009).

The fungi *Trichoderma* are living organisms which are dependent on many biological, physical and chemical factors which cause the biological effects of the fungi to be variable. Using organic material as carriers of *Trichoderma* could improve the survival of the antagonist in the soil. Organic waste materials and by-products from the agro-food processing industry can be used as carriers (Kancelista *et al.* 2013; Smolińska *et al.* 2014a; Smolińska *et al.* 2014b; Smolińska *et al.* 2016).

Biological control using antagonistic microorganisms provides great promise for the future management of diseases caused by soilborne pathogens because of their non-toxicity to humans and the environment (Köhl *et al.* 2011; Saraf *et al.* 2014; Hamza *et al.* 2016). However, this type of control shows reduced efficacy, especially when pathogen inoculum density is high. One reason for this situation is that soil represents a complex ecological niche, where a huge number of biological interactions occur between plants, bacteria, fungi and other organisms. Each organism struggles to survive in this highly competitive environment. The introduction of *Trichoderma* on organic carriers which serve as a source of food, should improve adaptation of the fungus to new conditions.

The aim of this work was to determine if selected *Trichoderma atroviride* TRS25 isolate with high chitynolitic activity, introduced into the soil on granulated waste material as an organic carrier, would reduce *S. sclerotiorum* sclerotia and *Ch. thielavioides* populations in the soil.

Materials and Methods

Microorganisms

The isolate *T. atroviride* TRS25 was obtained from the collection of the Microbiology Laboratory, Research Institute of Horticulture, Skierniewice, Poland. The morphological identification was confirmed by molecular classification carried by DNA barcoding, based on the sequences of ITS1 and ITS2 of the ribosomal RNA gene cluster and on the sequences of translation elongation factor 1 alpha (*tef*1), chitinase 18–5 (*chi*18–5), and RNA polymerase II subunit (*rpb*2) gene fragments (Oskiera *et al.* 2015). The isolate was kept frozen in glycerol at –80°C until use.

Sclerotinia sclerotiorum fungus was isolated from diseased lettuce and deposited in the Microbiology Laboratory collection. Production of sclerotia was conducted according to the method with sliced carrots of Knudsen *et al.* (1991). Plugs of fungal mycelium growing on potato dextrose agar (PDA, Merck) were transferred to 1 dm^3 flasks with sterilized sliced carrots. After 4 weeks of incubation at 25°C the sclerotia were harvested, rinsed with tap water and dried at room temperature.

Chalara thielavioides fungus deposited in the Microbiology Laboratory collection, was earlier isolated from diseased carrot roots. In this experiment the fungus was cultivated on PDA medium. After 7 days of incubation at 25°C, mycelium with spores from one Petri plate were scraped from the surface of the medium, suspended in 50 ml of sterile water and mixed in a blender.

Production of TRS25 on organic carrier

The production of the carrier was conducted according to patent application No. P.409340. The carrier was composed of organic waste materials – apple, strawberry, aronia, raspberry and currant pomaces. The materials were obtained from a factory producing fruit juices (Agropol Company, Potycz, Poland). The components were added at the proportion 1 : 1 : 1 : 1 : 1 (v/v), and 0.2 dm^3 tap water to 5 dm^3 mixture was added. The mixture was mixed and granulated in a granulator (P-300 Protechnika, Lukow, Poland). The granulates

were dried for about 10 days at 20–22°C and kept in plastic, perforated bags until use.

The spores of *T. atroviride* TRS25 were produced on PDA medium (Merck) on Petri plates. After 7 days of incubation at 25°C, mycelium with spores from one Petri plate were scraped from the surface of the medium and suspended in 50 ml of sterile water and mixed in a blender. The number of spores was counted with a hemocytometer under a light microscope (Olympus). Twenty-five ml of the suspension at the density 1×10^7–10^8 spores · ml^{-1} and 750 ml of water were added to 5 dm^3 of granulates. The granulates were incubated 18 days at room temperature. After this period 1 g of granulates contained about 10^9 cfu of *T. atroviride* TRS25.

Pot experiments

The experiments were conducted in plastic pots containing 10 dm^3 of raw sandy-loam soil (pH 7.3; salinity 0.32 g NaCl · l^{-1}; $N.NO_3$ – 53; P – 64; K – 107; Mg – 131; Ca – 2,090 mg · l^{-1} of soil). The soil was acquired from a conventional farm which grew crops. The following treatments were prepared:

1. Control (C) – soil without *S. sclerotiorum* and *Ch. thielavioides*.
2. Soil infested with *S. sclerotiorum* and *Ch. thielavioides* (P).
3. Soil + granulates without *T. atroviride* TRS25 (G).
4. Soil + granulates + *S. sclerotiorum* and *Ch. thielavioides* (G + P).
5. Soil + granulates overgrown with *T. atroviride* TRS25 + *S. sclerotiorum* and *Ch. thielavioides* (G + + TRS + P).
6. Soil + *T. atroviride* TRS25 suspension + *S. sclerotiorum* and *Ch. thielavioides* (TRS + P).

The treatments with pathogens contained *S. sclerotiorum* sclerotia and *Ch. thielavioides* inoculum. The *S. sclerotiorum* sclerotia were placed in 5-pocket nylon bags, one sclerotium/one pocket. Four bags were buried in each pot. Three ml of *Ch. thielavioides* suspension were added at the density 1×10^7spores · ml^{-1} per 1 dm^3 of soil.

The granulates were added to the soil at a concentration of 1% (w/v). Granulates were overgrown with *T. atroviride* TRS25 or without this isolate. Additionally, a treatment with *Trichoderma* fungi, without organic material was prepared. The *T. atroviride* TRS25 fungus grew on PDA medium. After 7 days of incubation the suspension of spores was prepared at the density 1×10^8 · ml^{-1}. One ml of the spore suspension was added per 1 dm^3 of the soil used in the pot experiment.

The pots were slightly covered with plastic foil to avoid drying and incubated for 6 months at room temperature. One treatment was prepared in three replications. The experiment was repeated.

Evaluation of *Sclerotinia sclerotiorum* sclerotia survival and *Chalara thielavioides* population in soil

The survival of *S. sclerotiorum* sclerotia was evaluated after 2 months of incubation. The bags were carefully removed from the soil and washed under running water. The sclerotia were taken from the pockets, counted, sterilized in 70% ethanol for 3 min, rinsed in distilled sterile water and placed separately on Petri plates (5 cm diameter) with PDA medium supplemented with antibiotics: streptomycin and rifampicin. After 8 days of incubation at 25°C the "healthy" sclerotia were counted. The sclerotia were able to produce a mycelium forming new sclerotia of *S. sclerotiorum*. Also, the number of sclerotia colonized by *Trichoderma* was counted.

The population of *Ch. thielavioides* in the soil was evaluated using carrot slices (discs) according to Kowalska and Smolińska (2003). The test was conducted three times: after 2, 4 and 6 months of incubation. Three doses of soil, 200 ml each, taken from each pot were placed into three Petri plates (18 cm diameter). Ten slices of carrots were placed on the soil surface of each plate. The slices were earlier sterilized in 70% ethanol and rinsed in distilled sterile water. The Petri plates were incubated at 24°C for 10 days. Then the slices were observed carefully. A carrot slice was classified as infected by *Ch. thielavioides* when black mycelium of *Ch. thielavioides* was observed. The mycelium isolated from the infected slices was observed under a microscope (Olympus) to confirm *Ch. thielavioides* identification. In this case the characteristic chlamydospores were observed.

Evaluation of *Trichoderma* propagules and other microorganisms in the soil

The analyses of microbial populations in the soil were conducted by a serial dilution method after 4 months. An aliquot of 100 µl each of dilution of soil sample was distributed on selective media. The following media were used: soil extract agar for evaluation of the total bacteria (Dhingra and Sinclair 1995); Gould medium (Gould *et al.* 1985) for fluorescent *Pseudomonas* enumeration; and Rose Bengal medium (Martin 1950) for *Trichoderma* fungi. The number of microorganisms was expressed as a number of colony forming unit (cfu) · g^{-1} of soil dry weight.

Data analysis

Significance of differences between means was established by one-way analysis of variance and the Newman-Keuls test at $p < 0.05$.

Results and Discussions

The management of some soilborne diseases is especially difficult because of the presence of survival structures (sclerotia, chlamydospores), which are very resistant and difficult to eradicate from the environment. Therefore, preventing their growth through the reduction or elimination of primary inoculum is essential for lowering disease incidence and severity. The studies presented in this paper demonstrate a biological method which may decrease the population of these pathogens in soil.

The conducted experiments showed that the amendment of soil with granulated organic materials, including apple, strawberry, aronia, raspberry and currant pomaces, had significant detrimental effects on *S. sclerotiorum* and *Ch. thielavioides*.

The population of *Ch. thielavioides* was estimated on the carrot disc biotest after 2, 4 and 6 months of soil incubation with organic amendments. In the first term of the treatment with the pathogens (P) the number of infected carrot discs was high – 71%. In treatments with the granulates – G + P and G + TRS + P the values were lower than the infected control – 49% and 69%, respectively but they were not statistically different (Fig. 1A). A significant detrimental effect of the granulates on *Ch. thielavioides* was observed after 4 and 6 months of incubation. Both granulates, overgrown with *T. atroviride* and granulates without *Trichoderma,* added to the soil inoculated with *Ch. thielavioides* decreased pathogen populations. In these treatments the amount of infected carrot discs was significantly lower than the inoculated control treatment. After 4 months (term II) in the treatment with pathogens (P) almost all carrot discs were infected – 94.3%, while in the treatment with granulates (G + P) and with *Trichoderma* overgrown granulates (G + TRS + P) – 42.3% and 52.3%, respectively (Fig. 1B). Similar significant differences were observed after 6 months (term III). In this case 72.3% of the carrot discs were infected in the treatment with the pathogens (P), while in the treatment with the granulates (G + P), only 33.3%, and in the treatment with the TRS25 overgrown granulates (G + TRS + P) – 30% (Fig. 1C).

Trichoderma atroviride TRS25 added in the form of spore suspension did not decrease the *Ch. thielavioides* population. Although the percent of infected carrot discs was slightly lower in terms I and II than in the infected control, the data were not statistically different. The results showed that the granulates added to the soil were efficient in decreasing the *Ch. thielavioides* population but the isolate *T. atroviride* TRS25 was not effective enough to eradicate this pathogen.

The granulated plant waste materials used in this study was composed of fruit pomaces (apple, strawberry, aronia, raspberry and currant). These by-products represent an important source of many biologically active compounds such as phenolic compounds and organic acids which have a wide range of activities e.g. antimicrobial (Djilas *et al.* 2009). Their activity depends on several physical, chemical and biological processes taking place in the soil (Gamliel *et al.* 2000; Bonanomi *et al.* 2010; Bonanomi *et al.* 2013).

Significant differences were observed in the treatments with *S. sclerotiorum*. Of the studied treatments

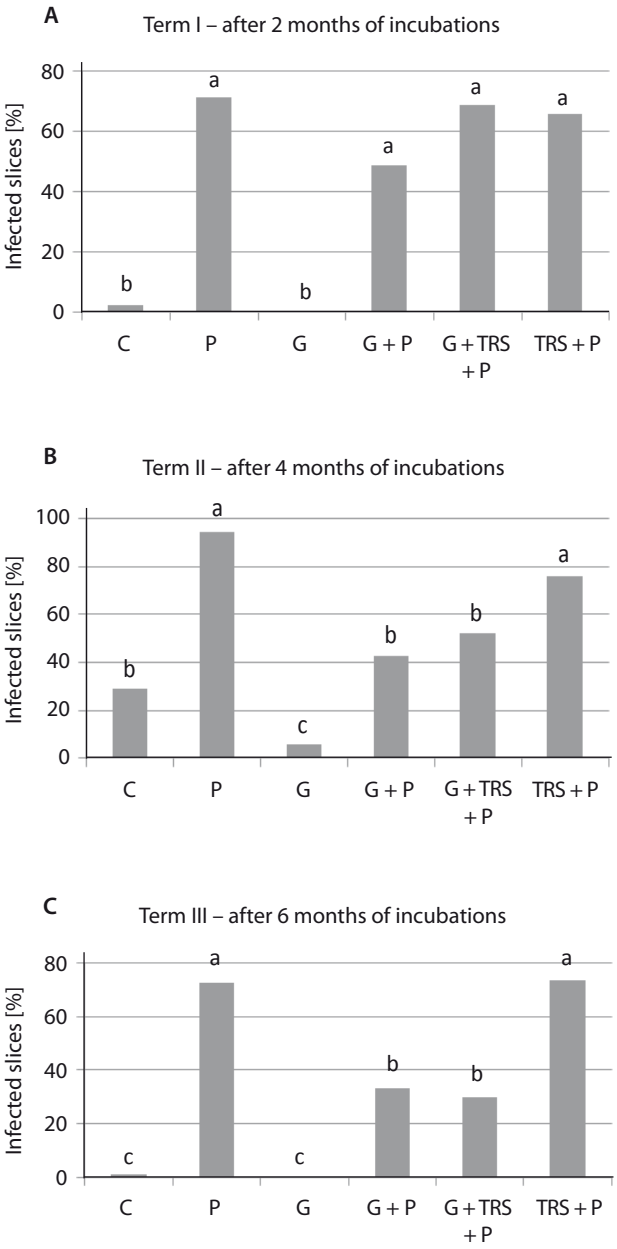

Fig. 1. Number of carrot slices infected by *Chalara thielavioides* obtained in the biotest after 2 (A), 4 (B) and 6 months (C) of incubation. C – control, P – pathogens, G – granulates, G + P – pathogens + granulates, G + TRS + P – granulates overgrown with TRS25 + pathogens, TRS + P – suspension of TRS25 + pathogens. Bars with the same letter are not significantly different according to Newman-Keuls test ($p < 0.05$). The presented results were obtained in the first experiment. The data obtained in the second experiment were similar

the fewest living sclerotia were obtained in the treatment with granulates overgrown with *T. atroviride* TRS25 (G + TRS + P), where the mean number of healthy sclerotia per one pot was 1.7, while in the control treatment – 14. In the treatment with the granulates without *Trichoderma* (G + P) and in the treatment with the *T. atroviride* suspension (TRS + P) an approximately 50% decrease of sclerotia survival was observed compared to sclerotia introduced into the soil, where the average values per one pot were 7 and 7.2 (Fig. 2A), respectively. Moreover, in the treatment with granulates overgrown with *T. atroviride* TRS25 (G + TRS + P), a mean of 16 sclerotia per pot obtained from soil were parasitized by *Trichoderma*, while in the treatment with *Trichoderma* spore suspension the average amount of parasitized sclerotia was only 3.7 (Fig. 2B).

The largest *Trichoderma* population in the soil was in the treatment with granulates overgrown with *T. atroviride* and estimated about 2.23×10^5 cfu $\cdot$ g^{-1} of soil dry weight, while in other treatments the values were much lower (Fig. 3A). The results showed that the *Trichoderma* population multiplied on organic material which had been introduced into the soil and survived in the environment for at least four months. The number of *Trichoderma* propagules in the soil where the fungus was introduced as a suspension was similar to the population in the treatments without the addition of *Trichoderma*. The observations are correlated with the authors' earlier studies in which it was observed that the granulates composed of organic waste materials were a good substrate for maintaining the population of *Trichoderma* in soil (Smolińska

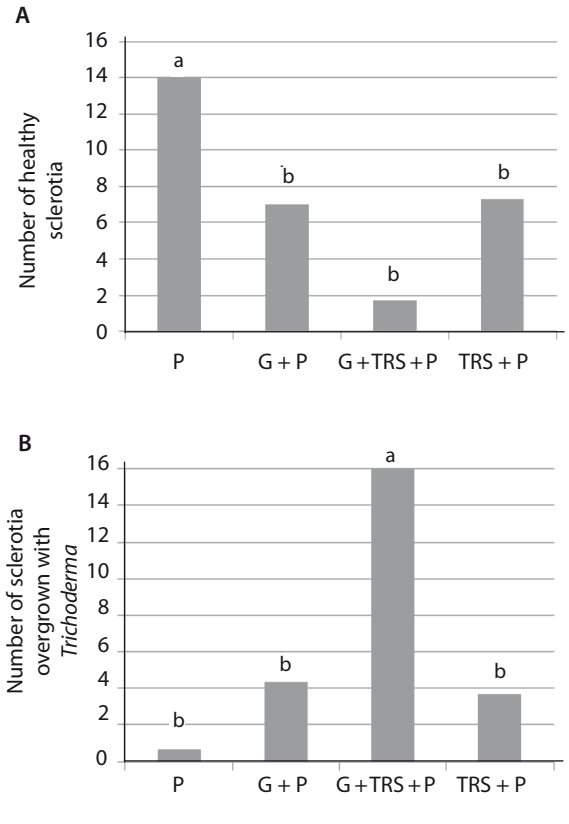

Fig. 2. Number of healthy *Sclerotinia sclerotiorum* sclerotia (A) and sclerotia overgrown with *Trichoderma* (B) obtained after 2 months of incubation in soil amended with the granulates and *Trichoderma atroviride* TRS25 isolate. P – pathogens, G + P – pathogens + granulates, G + TRS + P – granulates overgrown with TRS25 + pathogens, TRS + P – suspension of TRS25 + pathogens. Bars with the same letter are not significantly different according to Newman-Keuls test ($p < 0.05$). The presented results were obtained in the first experiment. The data obtained in the second experiment were similar

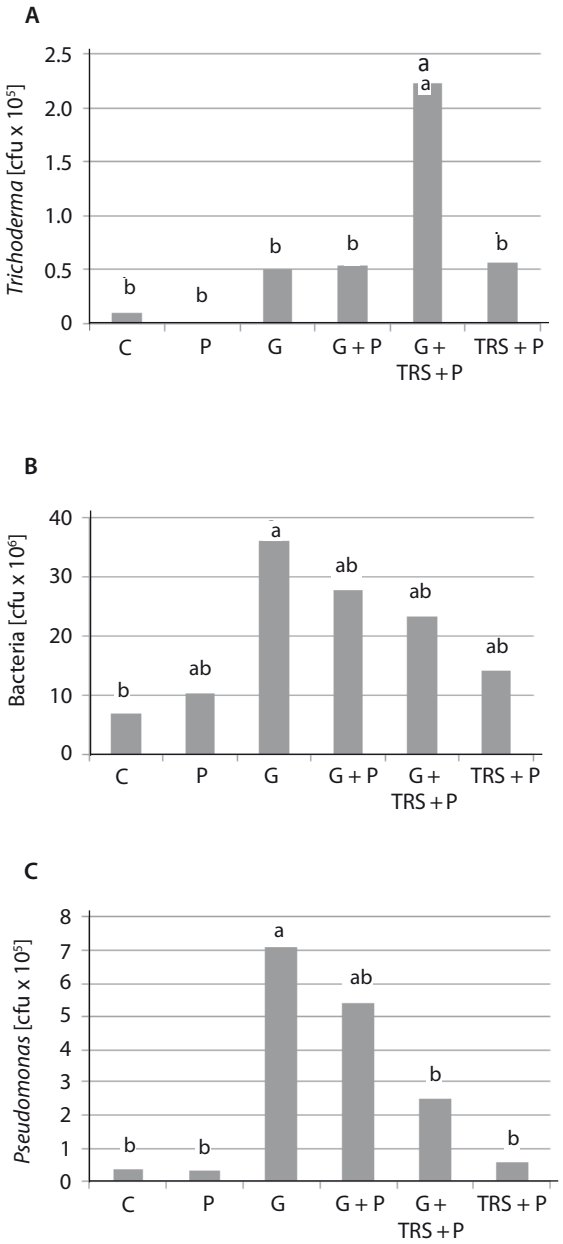

Fig. 3. The effect of granulates and *T. atroviride* TRS25 isolate on populations of *Trichoderma* sp. (A), the total number of bacteria (B) and population of *Pseudomonas* (C) in soil. C – control, P – pathogens, G – granulates, G + P – pathogens + granulates, G + TRS + P – granulates overgrown with TRS25 + pathogens, TRS + P – suspension of TRS25 + pathogens. Bars with the same letter are not significantly different according to Newman-Keuls test ($p < 0.05$). The presented results were obtained in the first experiment. The data obtained in the second experiment were similar

et al. 2014a; Smolińska *et al.* 2014b). Also studies conducted by Huang *et al.* (2005) showed that amending soil with a mixture of organic materials and biocontrol agent *T. virens*, enhanced the antagonistic effect of *Trichoderma* on plant pathogens. Mahdizadehnaraghi *et al.* (2015) observed that antagonistic fungi based bioformulations which contained the organic carrier were more effective in the control of white rot disease than those that contained the inorganic carrier.

The microbiological analysis of soil conducted after 4 months of incubation showed that the addition of granulated organic wastes had a positive influence on the population of microorganisms. In particular, an increase of the total number of bacteria was observed (Fig. 3B). The population of *Pseudomonas* was also higher in these treatments than in the treatments without organic waste material (Fig. 3C). Granulates added to the soil are good nutrient sources and are conducive to the growth and diversity of microbial communities. The positive effect of organic amendments on the growth and diversity of antagonistic microorganisms e.g. *Pseudomonas*, *Bacillus*, *Streptomyces* is well known (Scotti *et al.* 2015). These microorganisms increase the soil suppressiveness and often decrease the development of pathogens (Gamliel *et al.* 2000; Gilardi *et al.* 2016).

In conclusion, granulated waste materials composed of fruit pomaces overgrown with *T. atroviride* TRS25 and also without the antagonist could be used to eradicate the survival forms of plant pathogens *Ch. thielavioides* and *S. sclerotiorum*.

Acknowledgements

The studies were co-financed by the European Union through the European Regional Development Fund within the Innovative Economy Operational Program, 2007–2013, Priority 1.3.1. Project No. UDA-POIG.01.03.01-00-129/09 "Polish *Trichoderma* strains in plant protection and organic waste management".

References

Agrios G.N. 2005. Plant Pathology. 5th ed. Elsevier Academic Press, Burlington, USA, 922 pp.

Aleandri M.P., Chilosi G., Bruni N., Tomassini A., Vettraino A.M., Vannini A. 2015. Use of nursery potting mixes amended with local *Trichoderma* strains with multiple complementary mechanisms to control soil-borne diseases. Crop Protection 67: 269–278. DOI: https://doi.org/10.1016/j.oropro.2014.10.023

Bonanomi G., Antignani V., Capodilupo M., Scala F. 2010. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. Soil Biology and Biochemistry 42 (2): 136–144. DOI: https://doi.org/10.1016/j.soilbio.2009.10.012

Bonanomi G., Gaglione S.A., Incerti G., Zoina A. 2013. Biochemical quality of organic amendments affects soil fungistasis. Applied Soil Ecology 72: 135–142. DOI: https://doi.org/10.1016/j.apsoil.2013.06.007

Dhingra O.D., Sinclair J.B. 1995. Basic Plant Pathology Methods. 2nd ed. Lewis Publishers, Boca Raton, London-Tokyo, 434 pp.

Djilas S., Canadanović-Brunet J., Cetković G. 2009. By-products of fruits processing as a source of phytochemicals. Chemical Industry & Chemical Engineering Quarterly 15 (4): 191–202. DOI: https://doi.org/10.2298/CICEQ0904191D

Druzhinina I.S., Seidl-Seiboth V., Herrera-Estrella A., Horwitz B.A., Kenerley C.M., Monte C.M., Mukherjee P.K., Zeilinger S., Grigoriev I.V., Kubicek C.P. 2011. *Trichoderma*: the genomics of opportunistic success. Natural Review of Microbiology 9 (12): 749–759. DOI: https://doi.org/10.1038/nrmicro2637

Eshel D., Regev R., Orenstein J., Droby S., Gan-Mor S. 2009. Combining physical, chemical and biological methods for synergistic control of postharvest diseases: A case study of Black Root Rot of carrot. Postharvest Biology and Technology 54 (1): 48–52. DOI: https://doi.org/10.1016/j.postharvbio.2009.04.011

Gamliel A., Austerweil M., Kritzman G. 2000. Non-chemical approach to soilborne pest management – organic amendments. Crop Protection 19 (8–10): 847–853. DOI: https://doi.org/10.1016/s0261-2194(00)00112-5

Geraldine A.M., Lopes F.A.C., Carvalho D.D.C., Barbosa E.T., Rodrigues A.R., Brandao R., Ulhoa C.J., Junior M.L. 2013. Cell wall-degrading enzymes and parasitism of sclerotia are key factors on field biocontrol of white mold by *Trichoderma* spp. Biological Control 67 (3): 308–316. DOI: https://doi.org/101016/j.biocontrol.2013.09.013

Gilardi G., Pugliese M., Gullino M.L., Garibaldi A. 2016. Effect of different organic amendments on lettuce fusarium wilt and on selected soilborne microorganisms. Plant Pathology 65 (5): 704–712. DOI: https://doi.org/10.1111/ppa.12460

Gould W.D., Hagedorn C., Bardinelli T.R., Zablotowicz R.M. 1985. New selective media for enumeration and recovery of fluorescent *Pseudomonads* from various habitats. Applied and Environmental Microbiology 49 (1): 28–32.

Hamza A., Mohamed A., Derbalah A. 2016. Unconventional alternatives for control of tomato root rot caused by *Rhizoctonia solani* under greenhouse conditions. Journal of Plant Protection Research 56 (3): 298–305. DOI: https://doi.org/10.1515/jppr-2016-0046

Hermosa R., Viterbo A., Chet I., Monte E. 2012. Plant beneficial effects of *Trichoderma* and of its genes. Microbiology 158 (1): 1–25. DOI: https://doi.org/10.1099/mic.0.052274-0

Huang H., Erickson R.S., Chang Ch., Moyer J.R., Larney F.J., Huang J. 2005. Control of white mold of bean caused by *Sclerotinia sclerotiorum* using organic soil amendments and biological agents. Plant Pathology Bulletin 14: 183–190.

Johnson D.A., Atallah Z.K. 2014. Disease cycle, development and management of Sclerotinia stem rot of potato. American Journal of Plant Sciences 5 (25): 3717–3726. DOI: https://doi.org/10.4236/ajps.2014.525388

Kancelista A., Trill U., Stempniewicz R., Piegza M., Szczech M., Witkowska D. 2013. Application of lignocellulosic waste materials for the production and stabilization of *Trichoderma* biomass. Polish Journal of Environmental Studies 22 (4): 1083–1090.

Khaledi N., Taheri P. 2016. Biocontrol mechanisms of *Trichoderma harzianum* against soybean charcoal rot caused by *Macrophomina phaseolina*. Journal of Plant Protection Research 56 (1): 21–31. DOI: https://doi.org/10.1515/jppr-2016-0004

Knudsen G.R., Eschen D.J. Dandurand L.M., Bin L. 1991. Potential for biocontrol of *Sclerotinia sclerotiorum* through colonization of sclerotia by *Trichoderma harzianum*. Plant Disease 75 (5): 466–470. DOI: https://doi.org/10.1094/pd-75-0466

Kora C., McDonald M.R., Boland G.J. 2005. Epidemiology of sclerotinia rot of carrot caused by *Sclerotinia sclerotiorum*.

Canadian Journal of Plant Pathology 27 (2): 245–258. DOI: https://doi.org/10.1080/07060660509507222

Kowalska B., Smolińska U. 2003. Comparison of media used to isolate *Chalara elegans* and *Ch. thielavioides* from soil. Bulletin of the Polish Academy of Science 51 (2): 103–111.

Köhl J., Postma J., Nicot P., Ruocco M., Blum B. 2011. Stepwise screening of microorganisms for commercial use in biological control of plant-pathogenic fungi and bacteria. Biological Control 57 (1): 1–12. DOI: https://doi.org/10.1016/j.biocontrol.2010.12.004

Mahdizadehnaraghi R., Heydari A., Zamanizadeh H.R., Rezaee S., Nikan J. 2015. Biological control of garlic (*Allium*) white rot disease using antagonistic fungi-based bioformulations. Journal of Plant Protection Research 55 (2): 136–141. DOI: https://doi.org/10.1515/jppr-2015-0017

Martin J.P. 1950. Use of acid, rose Bengal and streptomycin in the plate method for estimating soil fungi. Soil Science 69 (3): 215–232. DOI: https://doi.org/10.1097/00010694-195003000-00006

Matroudi S., Zamani M.R., Motallebi M. 2009. Antagonistic effects of three species of *Trichoderma* sp. on *Sclerotinia sclerotiorum*, the causal agent of canola stem rot. Egyptian Journal of Biology 11: 37–44.

McQuilken M.P., Mitchell S.J., Budge S.P., Whipps J.M., Fenlon J.S., Archer S.A. 1995. Effect of *Coniothyrium minitans* on sclerotial survival and apothecial production of *Sclerotinia sclerotiorum* in field-grown oilseed rape. Plant Pathology 44 (5): 883–896. DOI: https://doi.org/10.1111/j.1365-3059.1995.tb02748.x

McQuilken M.P., Chalton D. 2009. Potential of biocontrol of sclerotinia rot of carrot with foliar sprayers of Contans WG (*Coniothyrium minitans).* Biocontrol Science and Technology 19 (2): 229–235. DOI: https://dx.doi.org/10.1080/09583150802635549

Monfil V.O., Casas-Flores S. 2014. Molecular mechanisms of biocontrol in *Trichoderma* spp. and their applications in agriculture. p. 429–453. In: "Biotechnology and Biology of *Trichoderma*" (V.K. Gupta, M. Schmoll, A. Herrera-Estrella, R.S. Upadhyay, I. Druzhinina, M.G. Tuohy, eds.). Elsevier. USA, 549 pp. DOI: https://doi.org/10.1016/b978-0-444-59576-8.00032-1

Oskiera M., Szczech M., Bartoszewski G. 2015. Molecular identification of *Trichoderma* strains collected to develop plant growth-promoting and biocontrol agents. Journal of Horticultural Research 23 (1): 75–86. DOI: https://doi.org/10.2478/johr-2015-0010

Paulin-Mahady A.E., Harrington T.C., McNew D. 2002. Phylogenetic and taxonomic evaluation of *Chalara*, *Chalaropsis*, and *Thielaviopsis* anamorphs associated with *Ceratocystis*. Mycologia 94 (1): 62–72. DOI: 10.2307/3761846

Saraf M., Pandya U., Thakkar A. 2014. Role of allelochemicals in plant growth promoting rhizobacteria for biocontrol of phytopathogens. Microbiological Research 169 (1): 18–29. DOI: 10.1016/j/micres.2013.08.009

Scotti R., Bonanomi G., Scelza R., Zoina A., Rao M.A. 2015. Organic amendments as sustainable tool to recovery fertility in intensive agricultural systems. Journal of Soil Science and Plant Nutrition 15 (2): 333–352. DOI: https://doi.org/10.4067/s0718-95162015005000031

Shafique H.A., Sultana V., Ehteshamul-Haque, Athar M. 2016. Management of soil-borne diseases of organic vegetables. Journal of Plant Protection Research 56 (3): 221–230. DOI: https://doi.org/10.1515/PPR-2016-0043

Smolińska U., Gołębiewska E., Kowalska B., Kowalczyk W., Szczech M. 2014a. Materiały odpadowe jako nośniki antagonistycznych grzybów *Trichoderma* [Waste materials as growing media for antagonistic *Trichoderma* fungi]. Inżynieria i Ochrona Środowiska 17 (1): 5–20 (in Polish)

Smolińska U., Kowalska B., Kowalczyk W., Szczech M. 2014b. The use of agro-industrial waste as carriers of *Trichoderma* fungi in the parsley cultivation. Scientia Horticulturae 179: 1–8. DOI: https://doi.org/10.1016/j.scienta.2014.08.023

Smolińska U., Kowalska B., Kowalczyk W., Szczech M., Murgrabia A. 2016. Eradication of *Sclerotinia sclerotiorum* sclerotia from soil using organic waste materials as *Trichoderma* fungi carriers. Journal of Horticultural Research 24 (1): 101–110. DOI: https://doi.org/10.1515/johr-2016-0012

Weber R.W.S., Tribe H.T. 2004. Moulds that should be better known: *Thielaviopsis basicola* and *T. thielavioides*, two ubiquitous moulds on carrots sold in shops. Mycologist 18 (1): 6–10. DOI: https://doi.org/10.1017/S0269915X04001028

Zeng W., Wang D., Kirk W., Hao J. 2012. Use of *Coniothyrium minitans* and other microorganisms for reducing *Sclerotinia sclerotiorum*. Biological Control 60 (2): 225–232. DOI: https://doi.org/10.1016/j.biocontrol.2011.10.009