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ERADICATION OF *SCLEROTINIA SCLEROTIORUM* SCLEROTIA FROM SOIL USING ORGANIC WASTE MATERIALS AS *TRICHODERMA* FUNGI CARRIERS

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ABSTRACT

Sclerotinia sclerotiorum (Lib.) de Bary is considered as one of the most harmful soilborne pathogens, which reduces productivity of horticultural crops. Currently used chemical or biological methods for the eradication of *S. sclerotiorum* from a soil are not very effective. The aim of this study was to evaluate the possibility of eradication of *S. sclerotiorum* sclerotia from a soil using the *Trichoderma* isolates, which were multiplied on the organic carriers prepared from agro-industrial wastes and by-products: WsA (wheat straw + apple pomaces), WsP (wheat straw + potato pulp) and T-GRAN (dry onion rind, apples and strawberry pomaces, rapeseed meal). The results showed that soil amendment with organic materials overgrown with the *Trichoderma* fungi had a significant reducing effect on *S. sclerotiorum*. Especially effective was the carrier WsA overgrown with *T. virens* TRS114, which completely prevented the survival of sclerotia of *S. sclerotiorum* regardless of the dose of application. Less effective was the WsP carrier. However, addition WsP overgrown with *T. atroviride* TRS40 at the 5% w/v, resulted in survival only 6.7% of sclerotia. In the greenhouse experiments with lettuce, the application of granulates T-GRAN into the soil had different impact on *S. sclerotiorum* depending on the conditions to the pathogen development. In conducive conditions, an addition of the organic substances without *Trichoderma* significantly decreased the yield of lettuce plants. A positive effect on the growth of plants was observed after the application of T-GRAN overgrown with *Trichoderma*.

Key words: *Sclerotinia sclerotiorum*, *Trichoderma*, sclerotia, organic wastes

INTRODUCTION

The fungus *Sclerotinia sclerotiorum* (Lib.) de Bary is worldwide distributed pathogen, which causes loss in the production of many crop species (Kora et al. 2003; Fernando et al. 2004). In the recent years, a growing problem with *S. sclerotiorum* was observed because of the specialization of agricultural production and the absence of effective methods of soil fumigation.

Spread of this disease is by the fragmentation of the mycelium, sclerotia and ascospores (Kora et al. 2003; Ordóñez-Valencia et al. 2015). Plants infected by this pathogen are covered with white, fluffy mycelium. Rot develops very quickly. Harmfulness of disease is high because the pathogen can survive in the soil for many years. Black sclerotia (compact mycelium) of irregular shape, of 0.5-1 cm in diameter, are formed in diseased plants. In conducive conditions for fungus development, they can germinate and produce mycelium directly or by apothecia.

Currently used chemical or biological methods for eradication of *S. sclerotiorum* from the soil are not effective. One of the method for the eradication of soilborne fungal pathogens from an infested soil is the application of antagonistic *Trichoderma* (Harman et al. 2004; Vinale et al. 2008; Hermosa et al. 2012; Yadav 2012; Geraldine et al. 2013). However, often effectiveness of this method is very low. The reason of a failure is mostly related to the fact that activity of *Trichoderma* is affected by many biotical and abiotical factors (Kredics et al. 2003). Mechanisms of *Trichoderma* activities towards pathogens were described in several papers (Knudsen et al.

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1991; Chet et al. 1998; Kaur et al. 2005; Huang at al. 2005; Zeilinger et al. 2007; Vinale et al. 2008; Druzhinina et al. 2011; López-Mondéjar et al. 2011). *Trichoderma* is highly competitive in the soil environment compared to other microorganisms. To be effective as biocontrol agent, the microorganism must be introduced into the environment in a sufficient quantity. Many microorganisms applied as biological control agents usually do not survive for long periods of time because of the intense competition for food by indigenous microorganisms. One of the methods to improve survival and development in soil may be the application of a beneficial microorganism with organic carriers (Coventry et al. 2006; Smolinska et al. 2014).

In this work, we used selected organic agro-industrial wastes and by-products as carriers of the antagonistic *Trichoderma* to eradicate *S. sclerotiorum* sclerotia from soil. The application of antagonistic fungi on organic media provides multiple benefits (Smolinska et al. 2014). First, this allows to introduce the *Trichoderma* in a large quantity into the soil. Second, the organic compounds are 'food base' for fungi, what allowed maintenance of population in the soil. Third, they contain valuable minerals which can be used by other microorganisms and plants. Additional advantage for the application of organic carriers is the allelopathic effect of compounds formed during decomposition in the soil. Their quantity and quality depend on several physical, chemical and biological processes taking place in the soil (Huang et al. 2002; Lattanzio et al. 2006; Bonanomi et al. 2011). Often they exhibit toxicity towards many microorganisms (Matthiessen & Kirkegaard 2006).

The aim of the study was to evaluate the possibility of eradication of *S. sclerotiorum* sclerotia from the soil using *Trichoderma* isolates, which were multiplied on the organic carriers prepared from agro-industrial wastes and by-products.

MATERIALS AND METHODS

Microorganisms used in experiments

The following *Trichoderma* isolates were used: *T. virens* TRS114 and TRS106; *T. atroviride* TRS40, TRS43 and TRS25; *T. harzianum* (sensu stricto) TRS59. The isolates were obtained from the collection of Microbiology Lab, Research Institute of Horticulture, Skierniewice. They were kept frozen in glycerol at −80 °C until use.

To produce sclerotia of *S. sclerotiorum*, the sliced carrots method was used (Knudsen et al. 1991). Plugs of fungal mycelium growing on the 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 black sclerotia were harvested, rinsed with tap water and dried at room temperature.

Organic waste materials and production of carriers

The following organic carriers were prepared: WsA (wheat straw + apple pomaces), WsP (wheat straw + potato pulp) and T-GRAN (dry onion rind, apples and strawberry pomaces, rapeseed meal). Materials were obtained from local farms and the factory producing fruit juices (Agropol Company, Potycz). The rapeseed meal was used as the commercial product (Ardex Company).

Production of WsA and WsP carriers. Wheat straw (pieces of about 0.5 cm) with dry apple pomace (WsA) or wheat straw with potato pulp (WsP) were mixed, 1:1 v/w. Next, the tap water was added (4 dm^3 of water to 1100 g of each mixture). The mixture was left for 18 h, at temperature at about 20-22 °C. After this time, the materials were left on screens for 30 min to remove excess of water. Then the media were heated at 100 °C for 1 h and placed after cooling in portions of 200 g in plastic bags (15 cm × 20 cm).

Production of T-GRAN granulates. To produce granulates T-GRAN (patent application no. P.402840), the method described by Smolinska et al. (2014) was used. The components of T-GRAN (dry onion rind, apple and strawberry pomaces, rapeseed meal) were mixed in the proportion 1:1:1:1 (v/v). After the addition of tap water, the mixture was granulated (Peleciarka P-300; Protechnika, Lukow). The granulates were dried for 5-10 days at ambient temperature (20-22 °C) and kept in plastic perforated bags until use.

Chemical analysis of organic waste material

The macro- and micronutrients (N, P, K, Ca, Mg, Na, S, Fe, Mn, Cu, Zn, B) with microwave digestion in the concentrated nitric acid closed system were measured (Ostrowska et al. 1991). Qualitative

and quantitative analyses of the above elements by the plasma spectrometry, atomic absorption spectroscopy and colorimetric 'segment flow system' were done (Antweiler et al. 1996). Total nitrogen through mineralization in concentrated sulphuric acid was determined (Walinga et al. 1995). The physical properties of organic materials were determined using the sand-kaolin equipment made by Eijkelkamp Co., dry oven and muffle furnace (C and ash content) according to the method described by Kaniszewski et al. (2012).

Multiplication of *Trichoderma* on T-GRAN, WsA and WsP carriers

Trichoderma spp. (*T. virens* TRS114 and TRS106; *T. atroviride* TRS40, TRS43 and TRS25; *T. harzianum* sensu stricto TRS59) were multiplied on the PDA (Merck) on Petri plates for 7 days at 25 °C. Mycelium with spores from one Petri plate was scratched from the surface of medium, suspended in 50 ml of sterile water and mixed in blender for about 10 s. The number of fungal spores was counted with the haemocytometer under the light microscope (Olympus).

Trichoderma isolates were cultivated on T-GRAN, WsA and WsP according to the method described by Smolinska et al. (2014). The organic materials were wetted with a tap water (150 ml·1 dm^{-3} of medium), then suspension of *Trichoderma* spores (5 ml·1 dm^{-3}; 10^8 cfu/ml) was added to each bag. To obtain the adequate oxygenation, the plastic bags were punched five times from each side with a needle. The inoculated carriers were kept for 12-14 days at a temperature of 20-22 °C. The quantity of *Trichoderma* propagules was evaluated with microbial methods using ST medium selective for *Trichoderma* (Chung & Hoitink 1990) and the Rose-Bengal medium (Martin 1950).

Effect of WsA and WsP carriers with *Trichoderma* on survival of *S. sclerotiorum* sclerotia in the soil (laboratory experiment)

The raw sandy-loam soil at moisture 70% (pH 7.4; salinity, 0.16 g NaCl dm^{-3}; 18 mg of $N\text{-}NO_3$; 56 mg of P; 57 mg of K; 81 mg of Mg; 1180 mg of Ca per liter of soil) was amended with WsA or WsP carriers, overgrowing with *Trichoderma* isolates: TRS114, TRS40, TRS106, at concentration of 1% and 5% w/w. After mixing the components, 3 kg of the soil were placed into a plastic pot (3 dm^3). Three nylon bags containing five pockets with five sclerotia in each were placed in the middle height of the pot. The control was the soil without organic amendments. Two pots were prepared per treatment. The pots were slightly covered to avoid drying and once a week sprayed with water. After one month, the bags were removed from soil, sclerotia were counted, sterilized in 70% ethanol for 3 min, rinsed in distilled sterile water and placed separately on Petri plates (5 cm in diameter) with PDA with antibiotics: streptomycin and rifampicin. After 8 days of incubation at 25 °C, the number of germination with mycelium sclerotia was counted. Also, the number of sclerotia colonized (parasitized) by *Trichoderma* was counted. Experiment was repeated once.

Effect of *Trichoderma* multiplied on T-GRAN carrier, on the lettuce growing in soil infested with *S. sclerotiorum*

Experiments were conducted to evaluate the effect of T-GRAN, overgrown with *Trichoderma*, on the growth of lettuce plants growing in the soil infested (or not) with *S. sclerotiorum*. Granulates T-GRAN overgrown with single *Trichoderma* isolates TRS43, TRS106, TRS25, TRS59 or with a mixture of two *Trichoderma* isolates, TRS25 + TRS106, TRS43 + TRS106, TRS59 + TRS43, were added to soil mixed with perlite (3:1), at a dose of 1% w/v. The soil with organic amendments (1.5 dm^3 for replication) was put into plastic pots. Then, the 6-week old lettuce seedlings 'Krolowa Lata' were planted, 1 seedling per pot. Immediately, five sclerotia of *S. sclerotiorum* were placed around each plant, 2 cm below the surface of the soil. Greenhouse experiments were established in random block design with six replications. Plants were managed according to the standard agricultural practice, including recommended nutrition and irrigation. After 4 weeks, the plants were cut and the mass of their upper parts was determined. Three experiments were conducted (Exp. I, Exp. II, Exp. III). In the experiment I, only the treatments 1-9 were evaluated.

The following treatments were applied:

1. Control I (soil with *S. sclerotiorum*)
2. T-GRAN + *S. sclerotiorum*
3. T-GRAN + TRS43 + *S. sclerotiorum*

4. T-GRAN + TRS106 + *S. sclerotiorum*
5. T-GRAN + TRS25 + *S. sclerotiorum*
6. T-GRAN + TRS59 + *S. sclerotiorum*
7. T-GRAN + TRS25 + TRS106 + *S. sclerotiorum*
8. T-GRAN +TRS43 + TRS106 + *S. sclerotiorum*
9. T-GRAN + TRS59 + TRS43 + *S. sclerotiorum*
10. Control II (soil without *S. sclerotiorum*)
11. T-GRAN
12. T-GRAN + TRS43
13. T-GRAN + TRS106
14. T-GRAN + TRS25
15. T-GRAN + TRS59
16. T-GRAN + TRS25 + TRS106
17. T-GRAN + TRS43 + TRS106
18. T-GRAN + TRS59 + TRS43

Evaluation of *Trichoderma* propagules in the soil

The population of *Trichoderma* propagules in the soil was assessed at the beginning of the experiment and after a month by a serial dilution method. An aliquot of 100 µl each of dilution of soil sample was distributed on the selective ST medium or the Rose-Bengal medium. The Petri plates were incubated at 25 °C for 5 days. Quantity of *Trichoderma* propagules was expressed as the number of colony forming units (cfu) in 1 g of dry soil.

Data analyses

The experiments with WsA and WsP were conducted twice. Experiments with T-GRAN were conducted three times. Significance of differences between means was established by one-way analysis of variance and the Newman-Keuls test at $p = 0.05$.

RESULTS

Chemical analysis of organic material

The carrier WsA has lower pH than WsP (5.3 and 6.8 respectively) and contained more nitrogen, iron, copper and boron than WsP (Table 1). Results from analysis of T-GRAN described by Smolinska et al. (2014) showed that granulates contained many valuable ingredients, especially a high concentration of phosphorus, iron, magnesium, zinc and manganese. The carriers obtained from organic wastes provided the components necessary for the growth of the *Trichoderma*. Analysis of *Trichoderma* propagules in the substrates overgrowing by fungus showed that the quantity was about 10^7-10^8 cfu·g^{-1} (results not presented).

Survival of *S. sclerotiorum* sclerotia in soil amended with WsA and WsP overgrowing with *Trichoderma* isolates

A month after addition of WsA or WsP overgrown with *Trichoderma*, the contents of sclerotia recovered from a soil ranged between 66.7% for WsA + TRS114 to 100% for WsA + TRS40, WsP + TRS114 and WsP + TRS106 (Table 2). In general, the higher amounts of sclerotia were obtained in the treatments with 1% addition of carrier compared to 5%. However, the differences were not significant.

Table 1. The chemical composition of WsA and WsP media

Media	pH	Total content (% of dry weight)						
		N	P	K	Ca	Mg	Na	S
WsA	5.3	0.74	0.05	0.37	0.22	0.07	0.009	0.09
WsP	6.8	0.57	0.04	0.32	0.26	0.07	0.008	0.06
		Total content (mg·kg^{-1})						
		Fe	Mn	Cu	Zn	B		
WsA		229	23.5	7.82	20.9	53.0		
WsP		150	30.3	4.56	29.5	31.6		
		Physical properties (%)						
		Dry weight	Organic matter	Ash content	C – total			
WsA		23.9	97.0	2.92	48.5			
WsP		19.3	96.8	3.15	48.4			

Table 2. Survival of sclerotia in soil amended with WsA and WsP media overgrown with *T. virens* TRS114, *T. virens* TRS106 and *T. atroviride* TRS40

Treatment	Sclerotia recovered from soil (%)	Live sclerotia recovered from soil (%) *	Sclerotia parasitized by *Trichoderma* (%)
Control	100 a	73.3 a	26.7 a
		1% amendment	
WsA+ TRS114	90 a	0 b	90.0 a
WsA +TRS40	100 a	16.7 ab	83.3 a
WsA +TRS106	96.7 a	10.0 ab	86.7 a
WsP +TRS114	96.7 a	40.0 ab	56.7 a
WsP +TRS40	96.7 a	16.7 ab	80.0 a
WsP+ TRS106	96.7 a	53.3 ab	43.3 a
		5% amendment	
WsA +TRS114	66.7 a	0 b	66.7 a
WsA +TRS40	96.7 a	0 b	96.7 a
WsA TRS106	70.0 a	6.7 b	63.3 a
WsP +TRS114	100 a	36.7 ab	63.3 a
WsP+ TRS40	80.0 a	6.7 b	73.3 a
WsP+ TRS106	100 a	43.3 ab	60.0 a

* The percentage of sclerotia was calculated in relation to the amount of sclerotia introduced to the soil. Means followed by the same letter in column do not differ significantly according to the Newman-Keuls test (p = 0.05). The statistical analysis was conducted separately for 1% and 5% amendment using the same control treatment.

Results presented in the Table 2 showed that WsA overgrown with TRS114 completely eliminated survival of *S. sclerotiorum* sclerotia regardless of a dose applied. Also, in the case of WsA + TRS40, added in the amount of 5%, live sclerotia were not recovered. Many of the sclerotia obtained were parasitised by *Trichoderma*. It means that on PDA and after 7 days of incubation, only *Trichoderma* mycelium was recovered. However, between the treatments, there were no significant differences in sclerotia parasitised by *Trichoderma.*

Much less efficient was WsP carrier. The WsP overgrown with TRS40 was the most effective in the destruction of sclerotia. After the addition of WsP + TRS40 at the 5% w/w, only 6.7% of sclerotia survived. The other two treatments (WsP + TRS114 and WsP + TRS106) decreased sclerotia survival to a lesser extent, 36.7% and 43.3%, respectively, compared to sclerotia introduced into the soil without organic amendments (Table 2). In general, the toxic effect towards sclerotia increased with the amount of organic materials (overgrown with *Trichoderma*) introduced into the soil.

Table 3. Population of *Trichoderma* in the soil amended with WsA and WsP media

Treatment	*Trichoderma* (log·cfu·g^{-1} dry soil)	
	Time „0"	After a month
	1% amendment*	
WsA + TRS114	4.69 b	4.55 ab
WsA + TRS40	5.30 a	4.80 a
WsA + TRS106	4.72 b	4.27 bc
WsP + TRS114	4.58 b	4.09 c
WsP + TRS40	4.77 b	4.02 c
WsP + TRS106	4.65 b	3.63 d
	5% amendment*	
WsA + TRS114	5.11 b	5.39 a
WsA + TRS40	5.68 a	5.44 a
WsA + TRS106	5.02 b	5.58 a
WsP + TRS114	5.30 b	4.91 a
WsP + TRS40	5.30 b	4.88 a
WsP + TRS106	5.03 b	4.72 a

*Amount of medium WsA or WsP overgrown with *Trichoderma* isolates, which were added to the soil. Means followed by the same letter in column do not differ significantly according to the Newman–Keuls test (p = 0.05). The statistical analysis was conducted separately for 1% and 5% amendment.

At the start of the experiment, the concentration of *Trichoderma* propagules in the soil amended with WsA and WsP carriers with *Trichoderma* strains balanced between 4.58 and 5.3 log·cfu·g^{-1} to 5.11 and 5.68 log·cfu·g^{-1} for 1% and 5% w/w addition, respectively (Table 3). At the end of the experiment, the quantity of *Trichoderma* propagules was slightly lower than that at the beginning in the most treatments, especially with the WsP carrier. Precise assessment of *Trichoderma* population was difficult because of the accumulation of fungi on organic particles and non-uniform distribution in the soil.

Effect of T-GRAN + *Trichoderma* on the lettuce growing in the soil infested with *S. sclerotiorum*

The addition of T-GRAN without *Trichoderma* to the soil infested with sclerotia, under conditions conducive for the pathogen, caused a strong infection of lettuce and decreased the mass of a head to 39.6% of the infested control (Fig. 1; Table 4, Exp. I). Amendments of the soil with T-GRAN + *Trichoderma* significantly stimulated the growth of lettuce plants in all treatments. In two subsequent experiments (Exp. II and Exp. III), infection of lettuce was low (results not presented), despite the fact that sclerotia of *S. sclerotiorum* were added into each pot. However, in all cases, the addition of T-GRAN overgrown with *Trichoderma* influenced positively the growth of lettuce, increasing mass of plants (279% of the control in treatment T-GRAN + TRS59 + *S. sclerotiorum*.

Table 4. Mass of lettuce head (% of the control) in the soil amended with T-GRAN + *Trichoderma* isolates, and infested (or not) with *S. sclerotiorum*

Treatment	Exp. I*	Exp. II**	Exp. III**
		Infested soil	
Control I (*with S. sclerotiorum)*	100 b	100 b	100 b
T-GRAN + *S. sclerotiorum*	39.6 c	221 a	182.4 a
T-GRAN + TRS43 + *S. sclerotiorum*	181 a	240 a	198.6 a
T-GRAN + TRS106 + *S. sclerotiorum*	208 a	242 a	153.3 a
T-GRAN + TRS25 + *S. sclerotiorum*	210 a	242 a	175.5 a
T-GRAN + TRS59 + *S. sclerotiorum*	207 a	279 a	175.2 a
T-GRAN + TRS25 + TRS106 + *S. sclerot*.	156 a	259 a	179.5 a
T-GRAN + TRS43 + TRS106 + *S. sclerot*.	203 a	231 a	151.1 a
T-GRAN + TRS59 + TRS43 + *S. sclerot*.	170 a	202 a	174.2 a
		Non-infested soil	
Control II	-	100 c	100 c
T-GRAN	-	148 bc	137 ab
T-GRAN + TRS43	-	228 ab	159 a
T-GRAN + TRS106	-	193 b	139 ab
T-GRAN + TRS25	-	191 b	123 b
T-GRAN + TRS59	-	181 b	141 ab
T-GRAN + TRS25 + TRS106	-	193 b	141 ab
T-GRAN + TRS43 + TRS106	-	194 b	156 ab
T-GRAN + TRS59 + TRS43	-	272 a	152 ab

*In the experiment I (Exp. I), strong infection of lettuce plants in the treatment with T-GRAN was observed.
** In the experiment II and experiment III, a weak infestation of all lettuce plants was observed in the combination infected with *S. sclerotiorum.*
Means followed by the same letter in column do not differ significantly according to the Newman–Keuls test (p = 0.05) for infested and non-infested soil separately.

Fig 1. Growth of lettuce plants in the soil infested with *S. sclerotiorum.* (A) Soil without organic amendments; (B) soil with T-GRAN; (C) T-GRAN + *T. atroviride* TRS43; (D) T-GRAN + *T. virens* TRS106. Results from Exp. I.

DISCUSSION

Farmers use organic wastes after composting or without processing. However, it is known that these easily degradable substances may have different effect on the soil microorganisms: a positive when they stimulate the plant-growth-promoting microbes or a negative when they increase the population of pathogens (Bonanomi et al. 2011; Klein et al. 2011; Smolińska & Kowalczyk 2014; Ascencion et al. 2015).

The results presented in this paper showed that the amendment of a soil with organic materials inoculated with *Trichoderma* fungi had significant effect on the reduction of *S. sclerotiorum*. Especially effective was the WsA carrier prepared from wheat straw and apple pomace and overgrown with the *T. virens* TRS114. This treatment completely eliminated the survival of sclerotia *S. sclerotiorum* regardless of a dose applied. Also, the other two isolates *T. atroviride* TRS40 and *T. virens* TRS106 decreased the amount of germinating sclerotia to 16.7% and 10.0%, respectively. However, complete sclerotia eradication was observed only at the 5% supplementation of WsA with *T. atroviride* TRS40. It is interesting that the same isolates of *Trichoderma* were much less effective when applied on WsP carrier prepared with the potato pulp. Apparently, it was visible with *T. virens* TRS114 isolate, which was very effective on WsA (0% of live sclerotia), but when they were added on the WsP carrier, the survival of sclerotia ranged between 36.7% and 40.0%.

Influence of the organic material on sclerotia survival was also detected after the assessment of the quantity of sclerotia parasitized by *Trichoderma*. In the case of WsA carrier, prepared on the base of wheat straw and apple pomace, the percentage of sclerotia parasitized by *Trichoderma* was always higher compared to WsP carrier with potato pulp, regardless of the kind of *Trichoderma* isolate used. The average percentage of sclerotia destroyed by *Trichoderma* at the dose of 1% was 86.7% for WsA and 60.0% for WsP. Although not so strong, the same tendency was observed at the dose of 5% where the average number of sclerotia overgrowing by *Trichoderma* was 75.6% for WsA and 65.5% for WsP.

It is difficult to explain why WsA + *Trichoderma* was more efficient in the eradication of sclerotia from soil than WsP + *Trichoderma*. Analysis of these two organic carriers showed that media with apple pomaces had pH 5.3 but with potato pulp pH was higher, 6.8. It is known that the optimum pH for the growth of fungi ranges between 4 and 6 (Paul & Clark, 2000), so in this aspect, the WsA carrier was favorable for *Trichoderma*. Another reason of different effect of WsA and WsP on sclerotia could be the food base for *Trichoderma*. Chemical analysis showed that the carrier with apple pomace was richer in macro- and microelements and contained more P, K, Fe and B than the carrier with potato pulp. Results in Table 3 showed that after one-month incubation, the population of *Trichoderma* was higher in the treatments with the WsA than with WsP. The carrier with apple pomace provided nutrients for the development of the fungus for a longer time than that with the potatoes pulp.

It is known that the composition of material and the conditions of decomposition are the major

determinants of the impact of organic material on plants and soil microorganisms and their interactions (Gamliel et al. 2000; Bonanomi et al. 2010; 2011). It is possible that addition of apple pomace was more detrimental for *S. sclerotiorum* than potato pulp because it contains high quantities of polyphenols (7000 µg/kg) (Kołodziejczyk et al. 2007), which antifungal activities was reported (Middleton 1996; Joshi et al. 2012). The antimicrobial properties of phenolic compounds from apple skin, the main ingredient of apple pomace, were proved by Alberto et al. (2006). It is known that some organic material added to the soil may have detrimental effect on survival of the fungal propagules (Huang et al. 2002; Smolińska et al. 2002; Bonanomi et al. 2011).

In the greenhouse experiments, the granulated mixture of organic materials: onion rind, apples and strawberry pomaces, rapeseed meal described as T-GRAN were used. T-GRAN contained all the compounds, carbon, nitrogen and mineral salts, essential for fungal growth (Smolinska et al. 2014). Granulates were overgrown with *Trichoderma* isolates TRS43, TRS106, TRS25, TRS59. Application of T-GRAN granulates into the soil had an impact on the lettuce plants, depending on the conditions – conducive or not, to the development of *S. sclerotiorum*. Despite the infection of the soil with the same number of sclerotia, only in the Exp. I, in the treatment with T-GRAN, significant infection of the lettuce was observed, and only in this experiment, the protective effect of T-GRAN + *Trichoderma* fungi against *S. sclerotiorum* was visible. In this conducive for disease condition, an addition of the organic substances without antagonistic fungi significantly decreased the yield of lettuce. In this case, the lack of competition for food increased population *S. sclerotiorum,* because the T-GRAN provided the substrate for a saprophytic growth of the pathogenic fungi. In two subsequent experiments, infestation of lettuce was minimal (results not presented). However, in all treatments, the positive effect of granulates T-GRAN overgrown with *Trichoderma* on lettuce plants was observed, regardless of the isolate type. The significant positive effect of the T-GRAN alone and T-GRAN overgrown with the *Trichoderma* was observed also on parsley seedlings in a growth chamber condition (Smolinska et al. 2014). In this paper, the T-GRAN stimulated the soil microbial activity. It could affect the health of plants indirectly through the increase in plant-growth-promoting microorganisms (Borneman & Becker 2007).

After analysis of about 2,500 experiments, Bonanomi et al. (2007) concluded that in about 45% experiments, after the addition of organic matter to soil, the significant decrease in plant diseases occurred; however, in about 20% experiments, the significant increase in disease incidence was observed. After the addition of organic amendments, the population of *S. sclerotiorum* increased by over 50% (Bonanomi et al. 2007).

The reason of application of organic materials into the field soil is improvement of soil structure and fertility and, on the other hand, help in utilization of organic wastes from agriculture production. It is important to find methods that would allow for safe application of organic material to infested field soil and to avoid the risk of increase of pathogen population.

The new regulations of the European Union introduced in 2008, the Code of Good Agricultural Practice, require farmers care about the humus. The use of organic wastes would allow farmers, with no livestock production, to meet the requirements of the new EU law.

The introduction of organic waste materials together with the antagonistic *Trichoderma* fungi to soil would have, in addition to the direct benefits to plants, the long-term positive impact to the soil. Because of the increase in the diseases caused by *S. sclerotiorum,* very important is the observation that these organic waste materials, used as carriers of *Trichoderma* spp., could decrease the population of *S. sclerotiorum* in a soil.

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