Archives of Environmental Protection
Vol. 43 no. 4 pp. 72–81

PL ISSN 2083-4772
DOI 10.1515/aep-2017-0039

DE GRUYTER OPEN

An assessment of adaptive and antagonistic properties of *Trichoderma* sp. strains in vegetable waste composts

Agnieszka Wolna-Maruwka[1]*, Tomasz Piechota[2], Alicja Niewiadomska[1], Jacek Dach[3], Magdalena Szczech[4], Małgorzata Jędryczka[5], Agnieszka A. Pilarska[6]

Poznan University of Life Sciences, Poland
[1]Department of General and Environmental Microbiology
[2]Department of Agronomy
[3]Instytute of Biosystems Engineering
[4]Research Institute of Horticulture in Skierniewice, Poland
[5]Institute of Plant Genetics, Polish Academy of Sciences, Poland
[6]Institute of Food Technology of Plant Origin, Poland

*Corresponding author's e-mail: amaruwka@up.poznan.pl

Keywords: moulds, compost, interaction, *Trichoderma*.

Abstract: The experiment consisted in monitoring the count of moulds and three selected *Trichoderma* sp. isolates (T1 – *Trichoderma atroviride*, T2 – *Trichoderma harzianum*, T3 – *Trichoderma harzianum*) in vegetable (onion and tomato) waste composted with additives (straw, pig manure). Additionally, the aim of the study was to determine the type of interaction occurring between autochthonous fungi isolated from composts after the end of the thermophilic phase and *Trichoderma* sp. strains applied in the experiment. Number of microorganisms was determined by the plate method, next the identification was confirmed. The rating scale developed by Mańka was used to determine the type of interactions occurring between microorganisms.

The greatest count of moulds in onion waste composts was noted in the object which had simultaneously been inoculated with two strains T1 – *T. atroviride* and T3 – *T. harzianum*. The greatest count of moulds was noted in the tomato waste composts inoculated with T2 – *T. harzianum* strain. Microscope identification revealed that *Penicillum* sp., *Rhizopus* sp., *Alternaria* sp. and *Mucor* sp. strains were predominant in onion waste composts. In tomato waste composts *Penicillium* was the predominant genus, followed by *Rhizopus*. The test of antagonism revealed the inhibitory effect of *Trichoderma* isolates on most autochthonous strains of moulds.

Tomato waste composts proved to be better substrates for the growth and development of *Trichoderma* sp. isolates. The results of the study show that vegetable waste can be used in agriculture as carriers of antagonistic microorganisms.

Introduction

One of major trends in handling waste and using it for energy purposes is to concentrate on reducing the amount of vegetable waste composts produced and on recycling it appropriately (Malińska et al. 2014).

According to Sołowiej (2007), agricultural production results in considerable amounts of waste, such as damaged or unused parts of plants, which often makes even as much as 20% of yield.

Onions (*Allium cepa* L.) are the second most important horticultural crop worldwide, after tomatoes. Over the past 10 years, onion production has increased by more than 25%. Accordingly, more than 500.000 tonnes of onion waste is produced annually in the European Union, mainly in Spain, the UK and Holland (FAO Statistics 2008).

In Poland there are numerous legal regulations which indicate that bioprocessing is a preferable method of handling biodegradable waste. For example, according to Council Directive 1999/31/EC, in 2020 Poland will have to reduce the amount of landfilled biodegradable communal waste by 65%. This legal act is an enormous challenge because landfilling is an essential method of handling waste in Poland (EU Council Directive 1999). Unprocessed waste is usually stored, which is a threat to the purity of underground and surface waters. According to Rosolak and Gworek (2006), landfills are environmentally unfriendly as they pollute the atmosphere with dust, gases, bacteria, odours and methane. They also attract birds and rodents.

Composting seems to be an effective and economical method of handling vegetable waste. This process gives a sanitarily safe product, which is highly useful as a fertiliser and meets the requirements of the Regulation of the Minister of the Environment on R10 recovery, issued on 20 January 2015 (Regulation of the Minister of the Environment 2015, Wolna-Maruwka et al. 2015).

The compost can be a perfect carrier for microorganisms promoting the growth and development of plants, such as fungi of the *Trichoderma* sp. genus (Smolińska et al. 2014), which undergo adsorption and overgrow composted organic matter. When they are entered into soil, they have a better chance to survive confrontation with the autochthonous soil microflora.

The use of such an innovative product, consisting of composted organic matter inoculated with strains of *Trichoderma* sp., for cultivation of selected plants strictly follows the current agricultural policy of Poland. On 1 January 2014 obligatory integrated plant protection was introduced in the EU countries. In Poland it was introduced in the Crop Protection Products Act (2013). According to the Act, integrated plant protection is a method of protecting plants from harmful organisms, which consists in using all plant protection methods available, especially non-chemical methods, minimising the health hazard to humans, animals and the environment.

There is a wide range of scientific reports proving the positive effect of *Trichoderma* sp. strains in promoting the growth and development of crops (Harman et al. 2004, Bal and Altintas 2006).

According to Szczech et al. (2008), fertiliser preparations containing *Trichoderma* sp. fungi are not common in the Polish market. Howell (2003) recommends that strains of active groups of microorganisms which are to be used as preparations should be acquired from the regions where they will be applied. It will ensure their quick adaptation to the soil environment and positive effect on the growth of plants.

The aim of the study was to assess the antagonistic properties of three strains of *Trichoderma* sp. on the autochthonous microflora in composts and to determine their adaptability to onion and tomato waste composts to be used for vegetable growing.

Material and methods

Experimental design

A composting experiment was set up in a field in spring 2013 and 2014. Tomato waste from greenhouse production, onion waste from mechanical peeling, wheat straw and pig manure were used in the study.

Composts used in the experiment were produced on a technical scale (in prisms with a capacity of about 20 tonnes). Tomato waste compost (mostly racemes and leaves) and onion waste compost (peel, rotten and small onions, etc.) was mixed with wheat straw (about 10%) and 5% of manure. The material was composted in prisms for about 6–7 weeks. When the thermophilic phase ended (prism temperature – about 25°C), each prism was divided into 3 parts (with two replicates). Samples for microbial analyses were collected and the prisms were inoculated with three strains of *Trichoderma* sp. (T1 – *T. atroviride*, T2 – *T. harzianum*, T3 – *T. harzianum*), which came from the collection of strains of the Institute of Horticulture in Skierniewice, Poland. An in vitro test had been conducted prior to inoculation to exclude the antagonistic effect of *Trichoderma* isolates applied in the experiment (Mańka 1974).

In order to prepare a spore suspension for inoculation of composts, isolates were proliferated on a solid PDA medium (Sigma Aldrich). After seven-day incubation at a temperature of 24°C the cultured *Trichoderma* sp. were scraped off with a sterile scalpel and suspended in sterile distilled water (500 ml). The suspension was homogenised in a laboratory mixer, which enabled separation of conidial spores from hyphae. The output concentration of individual spores in the obtained suspension was measured by means of a haemocytometer under a light microscope (Zeiss). The following results were noted: strain T1 – $2.9 \cdot 10^9$, T2 – $2.4 \cdot 10^9$, T3 – $3.0 \cdot 10^9$ per 1 ml. The composts were inoculated with a suspension of conidial spores concentrated at 10^4 per g w.m. of compost. Therefore, after calculation each prism of the composted material was respectively inoculated with 68.96 ml of T1 strain spore suspension, 83.33 ml of T2 isolate, 66.60 ml of T3 strain, 76.14 ml of a T1+T2 mixture and 67.78 ml a T1+T3 strain suspension. These conidial spore suspensions were mixed in 5 l of dechlorinated tap water and applied with a hand sprayer.

The following six combinations were used in the experiment:

TT1 – tomato waste compost inoculated with strain T1
TT2 – tomato waste compost inoculated with strain T2
TT1+T2 – tomato waste compost inoculated with strains T1 and T2
OT1 – onion waste compost inoculated with strain T1
OT3 – onion waste compost inoculated with strain T3
OT1+T3 – onion waste compost inoculated with strains T1 and T3

The experiment with compost inoculation with *Trichoderma* sp. strains was replicated twice, in spring 2013 and 2014.

Compost samples for microbial analyses were collected twice: one month and two months after the inoculation.

Microbiological analysis

The scope of experiments comprised determination (in five replications) of the total count of moulds and *Trichoderma* sp. The groups of microorganisms were cultured according to the plate method on solid substrates, using appropriate dilutions of soil solutions, expressed as $CFU \cdot g^{-1}$ of soil dry matter.

The count of moulds was determined on a medium prepared according to Martin (1950) with rose bengal and aureomycin added. Plates were incubated for 6 days at a temperature of 25°C. Colonies of moulds isolated from composts at the end of the thermophilic phase were inoculated to a PDA substrate (Sigma Aldrich) on the day of their inoculation with *Trichoderma* sp. isolates. Next, their systematic position was determined according to mycological keys and the percentage of individual genera in the entire population was calculated (Domsch et al. 1993).

The count of *Trichoderma* sp. strains was determined with the plate method, on a modified Martin's medium (Martin 1950) with chloramphenicol, streptomycin, metalaxyl and PCNB (pentachloronitrobenzene) added. The plates were exposed to visible light and incubated for 7 days at a temperature of 24°C. In order to confirm the systematic position of *Trichoderma* sp. in the *Trichoderma harzianum* or *Trichoderma atroviride* species the colonies were inoculated to the PDA substrate (Sigma Aldrich). They were initially identified with a microscope. Next, the identification was confirmed by means of in situ hybridisation (FISH), modified according to Amann et al. (1990), where 4% PFA (paraformaldehyde), 0.5% Triton solution, alcohol series (70%, 80%, 96%), 70% formamide solution and two probes, marked at end 3' with marker Cy3 (ACT CCC AAA CCC AAT GTG AA and ATA CCA AAC TGT TGC CTCGG) were applied (Siddiquee et al. 2010) (Figure 1).

The rating scale developed by Mańka (1974) was used to determine the type of interactions occurring between strains of autochthonous moulds isolated from the composts at the end of the thermophilic phase and *Trichoderma* sp. isolated applied in the research. The following factors were taken into consideration: the degree to which one colony surrounded another, the width of the inhibitory zone and growth inhibition. A positive result should be interpreted as an effect limiting the development of a particular autochthon (test fungus) by a selected *Trichoderma* sp. isolate (antagonist).

Before the experiment was set up, fungi had been proliferated on a PDA medium. Next, a sterile cork borer was used to cut discs of cultures (10 mm in diameter). The discs were transferred to the PDA medium and spaced at 30 mm from each other. Every day, for 8 days the mycelium diameter was measured in the dual-organism cultures and the type of interaction between the fungi was determined. The cultures were incubated at a temperature of 24°C. Single-organism cultures prepared in an analogical manner were used as control samples. All tests were replicated five times.

Chemical analysis

The chemical analysis of composts was conducted in the laboratory of the Department of Agronomy. The content of carbon was determined with the Tiurin method. The content of total nitrogen in the fresh weight of composts was measured with the Kjeldahl method by means of a Kjeltec 2200 System analyser (FOSS Tecator). The pH values of compost samples were determined in double distilled water. (pH_{H2O}). The content of dry weight of composts was measured by drying and weighing at a temperature of 105°C in the laboratory of the Institute of Biosystem Engineering.

Statistical analysis

Statistical analyses were conducted by means of Statistica 12.0 software (StatSoft Inc. 2012). Two-way analysis of variance was used to determine the significance of variation in the number of moulds under analysis, depending on the compost combination and term of analysis. Homogeneous subsets of means were identified by means of Tukey´s test at a significance level of $p = 0.05$.

Principal Component Analysis (PCA) was used to illustrate the dependence between the number of microorganisms and chemical parameters of compost.

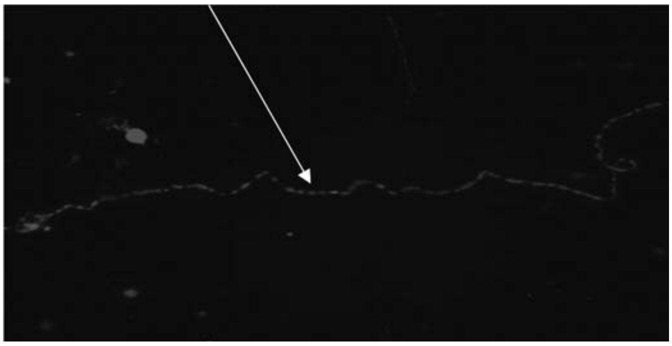

Fig. 1. Specific identification of whole fixed *Trichoderma harzianum* fungal hyphae with fluorescent oligonucleotide probes (FISH)

Results and discussion

The research was supposed to determine the possibility to use vegetable waste composts as substrates for the growth and development of fungi of the *Trichoderma* genus, which could be used for plant cultivation due to their phytosanitary and plant growth promoting properties (Bal and Altintas 2006).

Onion and tomato waste composts were used as carriers for three strains of *Trichoderma* sp. The decision about the choice of waste subjected to composting and inoculated with *Trichoderma* sp. isolates was influenced by the availability of waste because only the waste which is produced in large amounts can be used in agriculture.

Waste which is to be used as a substrate for microorganisms must be characterised by adequate chemical parameters (Smolińska et al. 2014) to guarantee optimal growth and development of microorganisms.

The analysis of chemical properties of the composts used in the experiments (Table 1) showed that the humidity in the combinations under study was similar and there were no statistically significant differences at p=0.05 at a particular term of measurement. The comparison of pH values of the composts in both experiments resulted in analogical observations. Apart from that, regardless of the type of experiment and compost, at the second term of analyses there was a statistically insignificant increase in the pH values in the combinations, which was most likely the effect of the application of *Trichoderma* sp. isolates.

This thesis is confirmed by findings made by Yedidia et al. (2001). As the authors observed, fungi of the *Trichoderma* sp. genus may increase the pH value of the substrate through the secretion of enzymes, such as cellulases, pectinases, xylanases and proteases. The study by Howell (2003) also confirmed that the enzymatic activity of *Trichoderma* sp. increased the pH of the substrate.

The analysis of the growth and development of moulds applied to the composts in experiment no. I revealed that in most cases the count of the moulds was increasing in a statistically significant way, i.e. at p=0.05 (Figures 2 and 3), as the experiment was progressing. The mycological assessment of the composts in experiment II showed a statistically significant decrease in the count of these microorganisms in most of the variants. The statistically significant increase in the proliferation of moulds in the composts at the end term of analyses (experiment I) arouses some controversy, because in general fungi prefer acidic or neutral pH of the substrate, whereas the pH value of the composts varied from 8.45 to 8.91.

However, as results from the study by Skowron et al. (2011), moulds also exhibit tolerance to alkaline pH. The authors observed that the count of these microorganisms reached the order of $1.50 \cdot 10^4$ CFU in composted sewage sludge when the pH value was 8.40.

The principal component analysis (PCA) did not confirm the influence of the pH value on the dynamics of growth of moulds either in the tomato composts or in the onion ones

(Figure 4). However, it proved the positive dependence between the count of moulds and humidity of compost combinations.

Compost humidity is a basic parameter influencing normal development of microorganisms. According to Malińska et al. (2014), in a mature compost it should amount to 50–60%.

The analysis of the count of moulds in the onion waste composts showed that when the experiment finished, both in the first and second experiment the greatest count of moulds was observed in the combination which had simultaneously been inoculated with two *Trichoderma* strains T1 and T3. As

Table 1. Properties of composts used in experiments

Properties	Kind of compost					
	TT1	TT2	TT1+T2	OT1	OT3	OT1+T3
Experiment I 1 month after inoculation						
pH_{H2O} value	8.01 ns	8.18 ns	7.99 ns	8.24 ns	8.34 ns	8.17 ns
humidity (%)	45.19 ab	45.43 ab	47.88 a–f	47.56 a–f	46.45 a–f	49.34 d–f
Experiment I 2 months after inoculation						
pH_{H2O} value	8.78 ns	8.47 ns	8.76 ns	8.91 ns	8.45 ns	8.67 ns
humidity (%)	54.36 h	59.65 g	50.98 ef	50.71 ef	48.11 a–f	54.66 g
Experiment II 1 month after inoculation						
pH_{H2O} value	8.18 ns	8.14 ns	8.34 ns	8.19 ns	8.26 ns	8.35 ns
humidity (%)	44.78 a	47.56 a–e	45.90 a–c	50.67 ef	49.56 d–f	45.89 a–c
Experiment II 2 months after inoculation						
pH_{H2O} value	8.41 ns	8.58 ns	8.77 ns	8.74 ns	8.99 ns	8.67 ns
humidity (%)	47.99 c–f	50.12 ef	49.12 c–f	55.98 g	50.78 ef	48.32 b–h

Explanation: Means followed by the same letters do not differ significantly at *p*=005;
ns-no significant difference;
Combination: TT1 – tomato waste compost inoculated with strain T1, TT2 – tomato waste compost inoculated with strain T2, TT1+TT2 – tomato waste compost inoculated with strain T1 and T2, OT1 – onion waste compost inoculated with strain T1, OT3 – onion waste compost inoculated with strain T3 , OT1+OT3 – onion waste compost inoculated with strain T1 and T3.

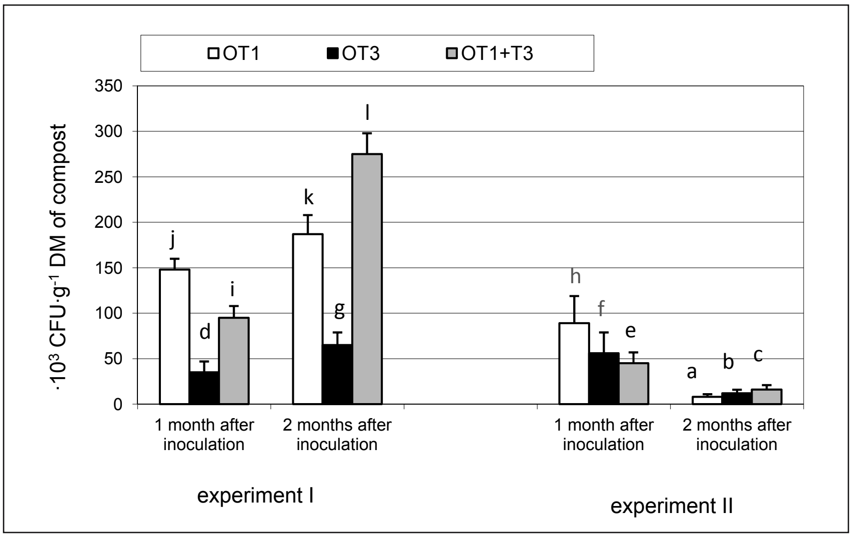

Fig. 2. The changes of total number of moulds in onion composts

Explanation: Means followed by the same letters do not differ significantly at *p*=0.05;
Combination: OT1 – onion waste compost inoculated with strain T1, OT3 – onion waste compost inoculated with strain T3, OT1+OT3 – onion waste compost inoculated with strain T1 and T3.

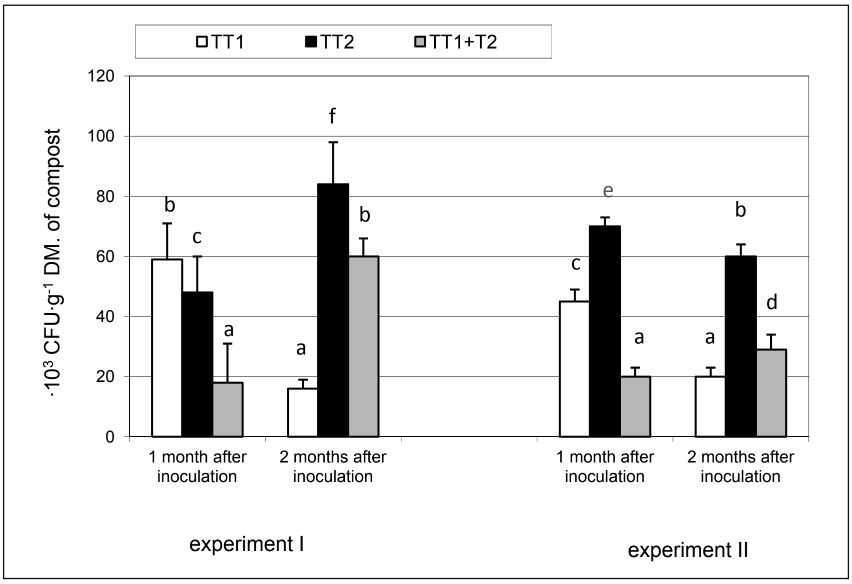

Fig. 3. The changes of total number of moulds in tomato composts

Explanation: Means followed by the same letters do not differ significantly at *p*=0.05;
Combination: TT1 – tomato waste compost inoculated with strain T1, TT2 – tomato waste compost inoculated with strain T2, TT1+TT2 – tomato waste compost inoculated with strain T1 and T2.

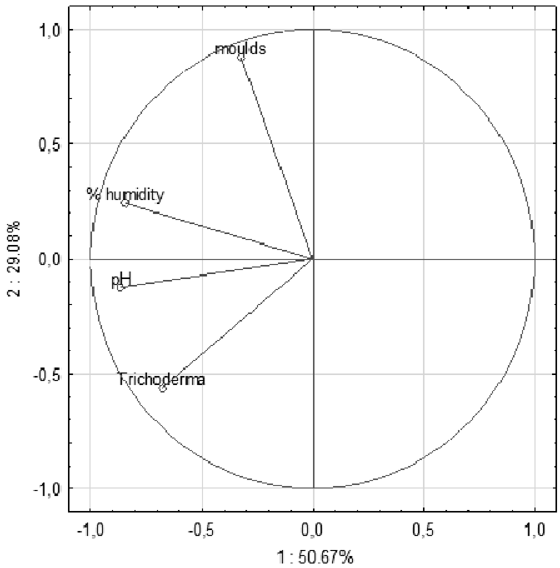

Fig. 4. Dependences between the number of moulds, *Trichoderma* sp, pH value and humidity (%) of applied in the experimental compost combinations at consecutive terms of analyses (PCA)

far as the tomato waste composts are concerned, the greatest count of moulds was noted in the variant inoculated with strain T2.

When we consider the count of moulds in the composts before the inoculation with *Trichoderma* sp. strains (Table 2), the research proved that regardless of the type of the *Trichodema* sp. isolate applied to the composts, in most of the experimental variants the inoculation noticeably decreased the total count of moulds (Figures 2 and 3). However, the principal component analysis (PCA), which accounted for a considerable amount of variability (more than 55%), did not prove that the applied *Trichodema* sp. isolates had statistically significant influence on limiting the growth and development of the autochthonous moulds in the composts.

The mycological analysis of the experimental variants conducted on the day of inoculation (Tables 3 and 4) proved that regardless of the type of composts, there was the highest percentage of fungi of the *Penicillium* genus, followed by the *Rhizopus* and *Aspergillus* genera. There was the lowest percentage of *Alternaria* sp. (2–4%), *Paecilomyces variotti* (2%) and *Cephalosporium* sp. (3%). Apart from that, the *Mucor* genus was isolated both from the tomato and onion waste composts, which in both cases amounted to 5% of the total population of the autochthonous microflora. The analysis of the composition of the moulds in the materials under

Table 2. The microbiological and chemical state of composts used in experiments (beginning of experiments)

Kind of compost	Total moulds number (10^3 cfu g^{-1} DM)	*Trichoderma* sp. (10^2 cfu g^{-1} DM)	pH_{H2O}	humidity (%)	C g·kg^{-1} DM	N g·kg^{-1} DM
Experiment I						
Tomato compost	100.78	2.46	7.78	40.55	344.81	17.93
Onion compost	133.78	–	7.99	45.89	353.80	21.73
Experiment II						
Tomato compost	93.33	3.99	8.01	38.99	406.00	20.22
Onion compost	198.45	–	7.67	41.67	447.10	23.11

Table 3. Isolates of moulds isolated from the tomato compost

Isolate	Percentage content (%)
Tomato compost – Experiment I	
Penicillum sp.	82
Rhizopus sp.	4
Trichoderma atroviride	14
Tomato compost – Experiment II	
Penicillium sp. (1)	16
Penicillium sp. (2)	28
Mucor sp.	5
Rhizopus nigricans	30
Rhizopus sp.	5
Trichoderma atroviride	16

Table 4. Isolates of moulds isolated from the onion compost

Isolate	Percentage content (%)
Onion compost – Experiment I	
Penicillum sp. (1)	48
Penicillium sp. (2)	36
Rhizopus sp.	7
Alternaria sp.	4
Mucor sp.	5
Onion compost – Experiment II	
Aspergillus niger	14
Rhizopus nigricans	20
Penicillium sp. (1)	19
Penicillium sp. (2)	35
Cephalosporium sp.	3
Paecilomyces variotti	2
Mucor sp.	5
Alternaria sp.	2

study proved that *T. atroviride* (14–16%) was also isolated from the tomato waste composts. These results confirm the findings made by Wojtkowiak–Gębarowska (2006), who observed common occurrence of *Trichoderma* in different environments (soil, plants, composts, manure).

Tests for antagonism proved that in both experiments selected *Trichoderma* sp. strains exhibited comparable antagonistic properties to moulds isolated from the tomato and onion waste composts. According to Dłużniewska (2004), *Trichoderma* sp. has numerous antagonistic properties. It is characterised by rapid growth, abundant sporulation, capacity to exist on many substrates and produce fungitoxic substances, easy use of available organic matter and inorganic compounds as well as parasitic capacity. Apart from that, the capacity of *Trichoderma* sp. to produce green pigment (Szczech et al. 2008) considerably facilitated estimation of the type of interaction between the antagonist and isolated microflora. This observation was confirmed in our study (Figure 5).

The analysis of the results presented in Tables 5 and 6 revealed that all the *Trichoderma* strains (T1, T2, T3) used in the experiments exhibited antagonistic properties to most of the moulds isolated from the plant material.

Isolate T1 – *T. atroviride* was characterised by the strongest biotic effects, which were particularly noticeable in experiment I.

According to Witkowska and Mai (2002), the phytosanitary properties of *Trichoderma* sp. result from the production of volatile and non-volatile compounds, where stronger effect is attributed to non-volatile secondary metabolites (enzymes and antibiotics). Also, according to Busko et al. (2008) and Howell (2003), fungi of the *Trichoderma* sp. owe their phytosanitary properties to antibiotics and antimetabolites (alamethicin, tricholine, peptaibols, viridine, gliovirin, gliotoxin, glisoprenin, heptelidic acid, α-pyrrole) and enzymes (cellulase, hemicellulase, xylanase, pectinase, β-1,3-glucanase, chitinase and protease), which degrade the mycelium.

According to Perek et al. (2013), combined activity of enzymes and antibiotics produced by microorganisms may result in greater antagonism than the individual effect of antibiotics or enzymes. Initial degradation of the cell wall by enzymes may facilitate penetration of antibiotics into the cells of other microorganisms.

In our study *Trichoderma* strains exhibited the weakest antagonism to *Mucor* sp. and *Rhizopus* sp. isolates as well as some of *Penicillium* sp. strains.

According to Piegza el al. (2009), weaker inhibitory properties of fungi of the *Trichoderma* genus may be caused by their weaker capacity to compete for food or place of colonisation, slower growth on a plate and poorer sporulation of *Trichoderma* isolates. In consequence, they are not capable of complete colonisation of space.

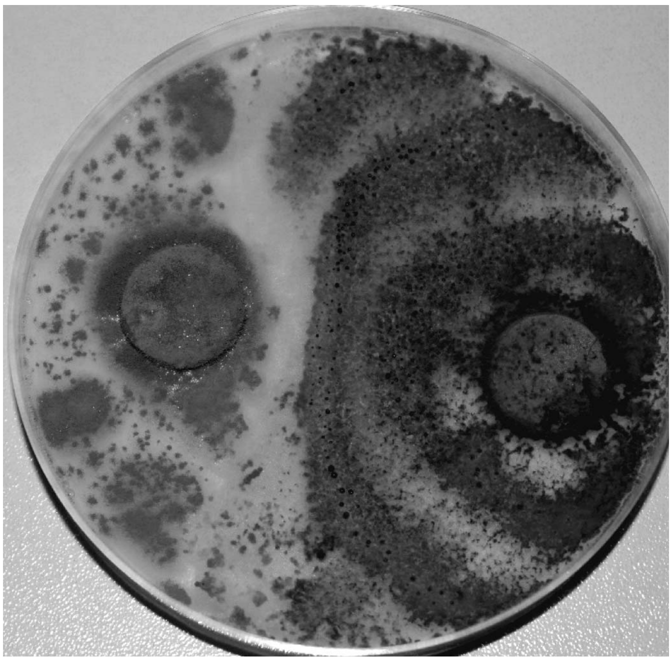

Fig. 5. Relationship between *Penicillium* sp. (2) isolate and *Trichoderma harzianum* – T3

Apart from that, Wawrzyniak and Waśkiewicz (2014) proved that some *Penicillium* species were able to produce antibiotics and mycotoxins, such as ochratoxin A and citrinin. According to the authors, these metabolites produced by some *Penicillium* strains have toxic effect on other microorganisms, including *Trichoderma*.

During the experiment, the count of *Trichoderma* sp. remained at 10^2 CFU g^{-1} DM, regardless of the compost type (Figures 6 and 7). All *Trichoderma* strains were applied to the composts at a conidia concentration of 10^4 g^{-1} of compost (Table 2). Thus, one month of incubation of composts reduced the count of microorganisms by two orders of magnitude. It is most likely that this phenomenon was related with the type of antagonistic effect between *Trichoderma* strains and the autochthonous microflora of the composts. This fact was confirmed by the aforementioned research findings. Dual-organism biotic tests conducted in this study proved the inhibitory effect of a few autochthonous strains isolated from both composts on the *Trichoderma* sp. strains applied in the composts (Tables 5 and 6).

The analysis of the count of *Trichoderma* sp. isolates (Figures 6 and 7) revealed that the tomato waste compost was a better carrier. It may have been caused by the mycological state of the compost (Table 2), where *T. atroviride* was part of the autochthonous microflora. Apart from that, it may have resulted from the chemical composition of the onion waste compost.

As results from the literature, active compounds of onion (allicin, allicepin, antifungal peptide, sulphur, fistulosin compounds) can destroy fungal cells, decreasing the oxygen uptake, reducing cellular growth, inhibiting the synthesis of lipids, proteins and nucleic acids, changing the lipid profile of the cell membrane and inhibiting the synthesis of the fungal cell wall (Phay et al. 1999, Wang and Ng 2004).

According to Smolińska et al. (2014a) and Cavalcante et al. (2008), agricultural waste can be successfully used as a carrier for fungi of the *Trichoderma* genus. The expansiveness of these microorganisms is caused by the possibility to use a wide range of compounds as a source of nourishment. According to Smolińska et al. (2014), the capacity of *Trichoderma* sp. to grow rapidly makes it one of the first fungi to colonise substrates after the process of fumigation.

The data provided in Figure 7 revealed that in both experiments, at the end of the experiment (the second month after the inoculation) the highest statistically significant count of *Trichoderma* was observed in the tomato waste compost which had simultaneously been inoculated with isolates T1 and T2.

The adaptive capacity of the strains applied individually into composts depended on the type of experiment. In experiment I isolate T1 – *T. atroviride* proliferated more intensely, whereas in experiment II – strain T2 – *T .harzianum*.

The growth and development of *Trichoderma* sp. was also diversified in the onion waste composts (Figure 4). In experiment I, at both terms the highest statistically significant count of isolate T3 – *T. harzianum* was noted, whereas in experiment II it was strain T1 – *T. atroviride*.

It is most likely that differences in the proliferation of *Trichoderma* isolates were caused by the type of interaction with the autochthonous microflora in a particular compost.

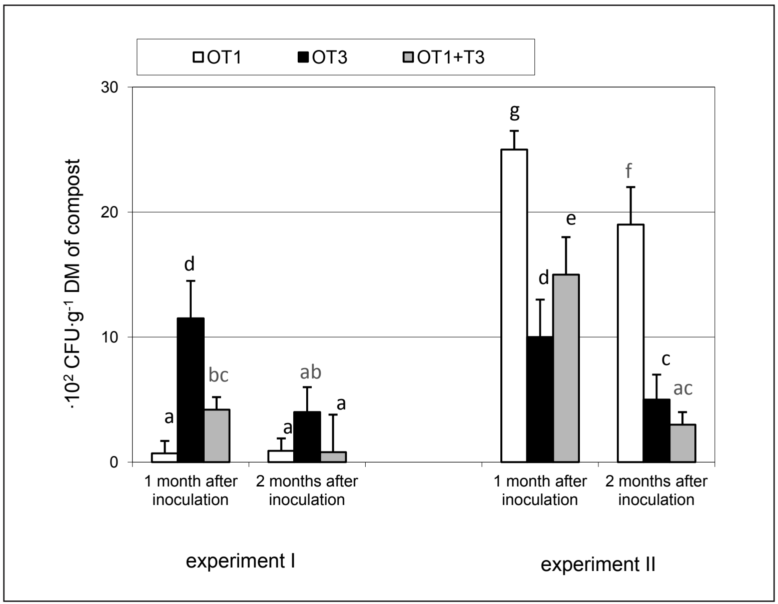

Fig. 6. The changes of *Trichoderma* sp. in onion composts
Explanation as Figure 2

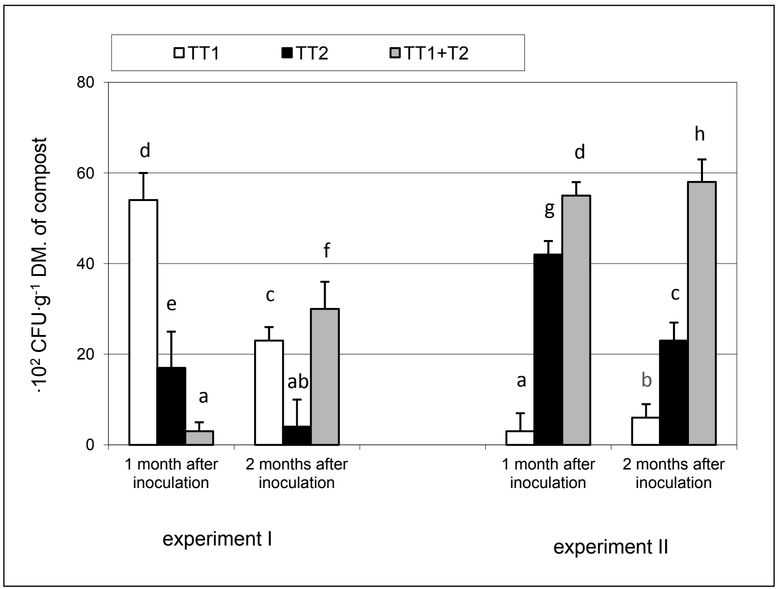

Fig. 7. The changes of *Trichoderma* sp. in tomato composts
Explanation as Figure 3

Table 5. Results of biotic test in dual cultures (tomato compost)

Tomato compost – Experiment I			
Isolate	*Penicillium* sp.	*Rhizopus* sp.	*Trichoderma atroviride*
T1	+4	+8	0
T2	+4	+6	0

Tomato compost – Experiment II						
Isolate	*Penicillium* sp. (1)	*Penicillium* sp. (2)	*Mucor* sp.	*Rhizopus nigricans*	*Rhizopus* sp.	*Trichoderma atroviride*
T1	+8	+8	+4	-4	+8	0
T2	+8	+8	+4	+4	+6	0

Explanation: A positive result means that the *Trichoderma* sp. isolate inhibited the growth of the autochthonous colony; 0 – no inhibition, 8 – total inhibition.

Table 6. Results of biotic test in dual cultures (onion compost)

Onion compost – Experiment I					
Isolate	*Penicillium* sp. (1)	*Penicillium* sp. (2)	*Rhizopus* sp.	*Alternaria* sp.	*Mucor* sp.
T1	+8	+8	+8	+8	+8
T3	+4	-6	+4	+6	+4

Onion compost – Experiment II								
Isolate	*Aspergillus niger*	*Rhizopus nigricans*	*Penicillium* sp. (1)	*Penicillium* sp. (2)	*Cephalosporium* sp.	*Paecilomyces variotti*	*Mucor* sp.	*Alternaria* sp.
T1	+4	-4	-4	-4	+4	+6	+8	+8
T3	-6	+4	+4	-6	+4	+4	+4	+6

Explanation as Table 5

Smolińska et al. (2014) also proved differences in the growth and sporulation of *Trichoderma* sp. isolates in substrates made from vegetable waste (carrot, beetroot, potato) and different types of straw. According to the authors, *Trichoderma* sp. strains differed in the mycelium growth intensity and sporulation on substrates with identical composition. The applied waste proved to be a good substrate for the growth of *Trichoderma* sp. Only barley straw added to substrates resulted in inhibition of the growth and sporulation of nearly all of the strains under analysis.

The results of our study let us conclude that the application of organic waste as a carrier for fungi of the *Trichoderma* genus had two advantages. First of all, it gives a possibility to handle waste which is harmful to the environment. Second of all, the use substrates containing antagonistic *Trichoderma* fungi will increase the population of useful microorganisms in soil and it will probably translate into the yield of crops.

Conclusions

1. The highest count of fungi in the onion waste composts was observed in the variant which had simultaneously been inoculated with two strains T1 – *T. atroviride* and T3 – *T. harzianum*. The greatest count of moulds in the tomato waste composts was noted in the combination with T2 – *T. harzianum* strain.
2. The onion waste compost proved to be a good carrier both for strain T1 – *T. atroviride*, and T3 – *T. harzianum* (which had been applied separately to the composts).
3. In the tomato waste composts the most intense proliferation of isolates was observed in the variant where two strains had been applied simultaneously, i.e. T1 – *T. atroviride* and T2 – *T. harzianum*.
4. *Penicillum* sp. strains were predominant in the onion waste composts. In total they made 84% of the population of microorganisms in experiment I. In experiment II Penicillium sp. isolates made 54% of the population, whereas *Aspergillus niger* and *Rhizopus nigricans* made 34%.
5. *Penicillium* was also the predominant genus in the tomato waste composts, where it amounted to 82% of the population of microorganisms in experiment I and 44% in experiment II.
6. The research proved that composted onion and tomato waste, which could be used for vegetable growing, was a good carrier for antagonistic strains of *Trichoderma* sp. It will increase the yield of crops by inhibiting the growth of plant pathogens.

Acknowledgements

The research was conducted as part of the project of the National Centre for Research and Development, No. UDA--POIG.01.03.01-00-129/09-09 'Polish Strains of *Trichoderma* in Plant Protection and Organic Waste Handling'.

References

Amann, R.I., Krumholz, L. & Stahl, D.A. (1990). Fluorescent--oligonucleotide probing of whole cells for determinative, phylogenetic and environmental studies in microbiology, *Journal of Bacteriology*, 172, 2, pp. 762–770.

Bal, U. & Altintas, S. (2006). A positive side effect from Trichoderma harzianum, the biological control agent: Increased yield in vegetable crops, *Journal of Environmental Protection and Ecology,* 7, 2, pp. 383–387.

Busko, M., Chełkowski, J., Popiel, D. & Perkowski, J. (2008). Solid substrate bioassay to evaluate impact of Trichoderma on trichothecene mycotoxin production by Fusarium species, *Journal of the Science of Food and Agriculture,* 88, 3, pp. 536–541.

Cavalcante, R.S., Lima, H.L.S., Pinto, G.A.S., Gava, C.A.T. & Rodrigues, S. (2008). Effect of moisture on Trichoderma conidia production on corn and wheat bran by solid state fermentation, *Food and Bioprocess Technology*, 1, 1, pp. 100–104.

Crop Protection Products Act of 8 March 2013, Official Journal 2013 Pos. 455, pp. 1–53. (in Polish)

Dłużniewska, J. (2004). The influence of incubation temperature on the development and biotic relations between Trichoderma spp. and pathogenic fungi, *Acta Scientiarum Polonorum, Agricultura*, 3, 2, pp. 257–262. (in Polish)

Domsch, K.H., Gams, W. & Anderson, T.H. (1993). *Compendium of soil fungi*, ed., Academic Press, San Francisco 1993.

EU Council Directive 1999/31/CE of 26 April 1999 on Waste Storage, EU Official Journal 1999, L182/1, pp. 228–246.

FAO Statistics,(2008). Productions, crops. (http://faostat.fao.org/site/567/default.aspx#ancor (15.02.2008)).

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I. & Lorito, M. (2004). Trichoderma species-opportunistic, avirulent plant symbionts, *Nature Reviews Microbiology,* 2, 1, pp. 43–56.

Howell, 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, 1 pp. 4–10.

Malińska, K., Zabochnicka-Świątek, M. & Dach, J. (2014). Effects of biochar amendment on ammonia emission during composting of sewage sludge, *Ecological Engineering*, 7, pp. 474–478.

Mańka, K. (1974). Fungal communities as criterion for estimating the effect of the environment of plant disease in Poland, *Zeszyty Problemowe Postępów Nauk Rolniczych,* 160, pp. 9–23. (in Polish)

Martin, J.P. (1950). Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi, *Soil Science*, 69, pp. 215–232.
Perek, A., Krzymińska, J. & Świerczyńska, I. (2013). Comparison of the antagonistic effect of Trichoderma spp. and yeasts on pathogenic Fusarium spp. in in vitro conditions, *Journal of Research and Applications in Agricultural Engineering*, 58, 4, pp. 99–103. (in Polish)
Phay, N., Higashiyama, T., Tsuji, M., Matsuura, H., Fukushi, Y., Yokota, A. & Tomita, F. (1999). An antifungal compound from roots of Welsh onion, *Phytochemistry*, 52, pp. 271–274.
Piegza, M., Stolaś, J., Kancelista, A. & Witkowska, D. (2009). The influence of fungi of the Trichoderma genus on the growth of pathogenic filamentous fungi in biotic tests on atypical sources of carbon, *Acta Scientiarum Polonorum, Biotechnologia*, 8, 1, pp. 3–14. (in Polish)
Regulation of the Minister of the Environment on R10 recovery, issued on 20 January 2015, Pos. 132. (in Polish)
Rosalak, M. & Gworek, B. (2006). The state and assessment of waste handling in Poland, *Ochrona Środowiska i Zasobów Naturalnych*, 29, pp. 71–84. (in Polish)
Siddiquee, S., Yusof, N.A., Salleh, A.B., Tan, S.G., Bakar, F.A. & Heng, L.Y. (2010). DNA hybridization based on Trichoderma harzianum gene probe immobilization on self-assembled monolayers on a modified gold electrode, *Sensors and Actuators B: Chemical*, 147, pp. 198–205.
Skowron, K., Olszewska, H. & Pawłowska, A. (2011). The effect of composting process on changes in quantity of yeast and mould fungi population, *Rocznik Ochrona Środowiska*, 3, 1, pp. 1485–1506. (in Polish)
Smolińska, U., Gołębiewska, E., Kowalska, B., Kowalczyk, W. & Szczech, M. (2014). Waste materials as carriers of antagonistic *Trichoderma* fungi, *Inżynieria i Ochrona Środowiska*, 17, 1, pp. 5–20. (in Polish)
Smolińska, U., Kowalska, B., Kowalczyk, W. & Szczech, M. (2014a). The use of agro-industrial wastes as carriers of Trichoderma fungi in the parsley cultivation, *Original Scientia Horticulturae*, 179, 24, pp.1–8.
Sołowiej, P. (2007) An example of using a compost prism as a low--temperature source of heat. Department of electrical and power engineering, University of Warmia and Mazury in Olsztyn, *Inżynieria Rolnicza*, 8, 96, pp. 247–249. (in Polish)
Szczech, M., Staniszek, M., Habdas, H., Uliński, Z. & Szymański, J. (2008). *Trichoderma* spp. The cause of green mold on polish mushroom farms, *Vegetable Crops Research Bulletin,* 69, pp. 105–114.
Wang, H.X. & Ng, T.B. (2004). Isolation of allicepin, a novel antifungal peptide from onion (Allium cepa) bulbs, *Journal of Peptide Science*, 10, 3, pp. 173–177.
Wawrzyniak, J. & Waśkiewicz, A. (2014). Ochratoxin A and citrinin production by Penicillium verrucosum on cereal solid substrates, *Food Additives and Contaminants: Part A,* 31, 1, pp. 139–148.
Witkowska, D. & Maj, A. (2002). Production of lytic enzymes by Trichoderma spp. and their effect on the growth of phytopathogenic fungi, *Folia Microbiologica*, 47, 3, pp. 279–282.
Wojtkowiak-Gębarowska, E. (2006). Mechanisms of controlling soil phytopathogens with fungi of the Trichoderma genus, *Postępy Mikrobiologii*, 45, 4, pp. 261–273. (in Polish)
Wolna-Maruwka, A., Mocek-Płóciniak, A., Głuchowska, K., Schroeter-Zakrzewska, A., Borowiak, K., Niewiadomska, A., Starzyk, J. & Dach, J. (2015). Assessment of the influence of composts on microbiological and biochemical parameters of substrates and the morphological traits of scarlet sage, *Archives of Environmental Protection*, 41(3), pp. 28–38.
Yedidia, I., Srivastava, A.K., Kupalnik, Y. & Chet, I. (2001). Effect of Trichoderma harzianum on microelement concentrations and increased growth of cucumber plants, *Plant and Soil*, 235, pp. 235–242.

Ocena właściwości adaptacyjnych i antagonistycznych szczepów Trichoderma sp. w kompostach z odpadów warzywnych

Streszczenie: Przeprowadzone doświadczenie polegało na monitorowaniu liczebności grzybów pleśniowych oraz trzech, wybranych izolatów *Trichoderma* sp. (T1 – *T. atroviride*, T2 – *T. harzianum*, T3 – *T. harzianum*) w kompostowanych wraz z dodatkami (słoma, obornik świński) odpadach warzywnych (odpady cebulowe i pomidorowe). Dodatkowo celem badań było określenie rodzaju interakcji zachodzącej między autochtonicznymi grzybami wyizolowanymi z kompostów, po ustaniu fazy termofilnej a zastosowanymi szczepami Trichoderma sp. Liczebność mikroorganizmów określano metodą płytkową, a następnie przeprowadzano identyfikację potwierdzającą. Rodzaj interakcji między mikroorganizmami określano stosując metodę Mańki. Najwyższą liczebność grzybów pleśniowych w kompostach wytworzonych na bazie odpadów cebulowych odnotowano w obiekcie zainokulowanym jednocześnie dwoma szczepami T1 – *T. atroviride* oraz T3 – *T. harzianum.* Z kolei w kompostach pomidorowych, w kombinacji z dodatkiem szczepu T2 – *T. harzianum*. Przeprowadzona identyfikacja mikroskopowa wykazała, że w kompostowanych odpadach cebulowych dominowały szczepy *Penicillum* sp., *Rhizopus* sp., *Alternaria* sp., *Mucor* sp. Z kolei w odpadach pomidorowych dominującym rodzajem okazał się *Penicillum*., a następnie *Rhizopus*. Przeprowadzony test na antagonizm wykazał, ponadto inhibicyjne wpływ izolatów *Trichoderma* w stosunku do większości autochtonicznych szczepów grzybów pleśniowych Komposty wytworzone z odpadów pomidorowych okazały się lepszym podłożem dla wzrostu i rozwoju izolatów Trichoderma sp. Wyniki uzyskane w pracy wskazują, że odpady warzywne można wykorzystać w rolnictwie jako nośniki mikroorganizmów antagonistycznych.