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ASSESSMENT OF INFLUENCE OF *TRICHODERMA* SP. ON THE SOIL SANITARY CONDITION AND THE YIELD OF NAPA CABBAGE

Summary

We conducted a two-year study to prove the antagonistic properties between three Trichoderma sp. strains entered into soil in compost carriers and plant pathogens of the Fusarium and Alternaria genera. Another aim of the study was to analyse the influence of these strains on the value of the biological fertility index (BIF) and the commercial yield of napa cabbage. Eleven combinations were used in the experiment, including a control variant, a variant with soil treated with a mineral fertiliser, one with manure and eight variants treated with tomato or onion waste composts. Some of them were inoculated with Trichoderma atroviride (T1) and/or T. harzianum (T2 and T3) isolates. Soil samples were collected at three terms of the investigations and they were subject to microbiological (the total count of moulds, Trichoderma sp., Fusarium sp. and Alternaria sp.) and enzymatic analyses (dehydrogenase and catalase activity). After harvesting the dry and fresh weight of napa cabbage leaves was measured. The total count of moulds and the commercial yield of napa cabbage were most strongly modified in the variant with the tomato waste compost inoculated with the T1 strain. The variants with the onion waste composts caused the greatest increase in the biological fertility index (BIF). The research showed that the phytosanitary properties of Trichoderma sp. resulted from the specificity of the species. The smallest count of Fusarium sp. was observed in the soil treated with the onion waste compost inoculated with the T1 strain. The smallest count of Alternaria sp. was noted after treatment with the tomato waste compost, which was simultaneously inoculated with two isolates, i.e. T1 and T2. Apart from that, the research proved that both types of composts were good carriers for Trichoderma sp. isolates. The tomato waste composts caused more intense proliferation of Trichoderma sp. in soil.
***Key words**: moulds, Trichoderma, compost, soil, cabbage*

OCENA WPŁYWU SZCZEPÓW *TRICHODERMA* SP. NA STAN SANITARNY GLEBY ORAZ PLONOWANIE KAPUSTY PEKIŃSKIEJ

Streszczenie

Przeprowadzono dwuletnie badania, których celem było wykazanie właściwości antagonistycznych trzech szczepów Trichoderma sp. wprowadzonych do gleby na nośnikach w postaci kompostów, w stosunku do patogenów roślinnych z rodzaju Fusarium i Alternaria. Ponadto celem badań była analiza wpływu wyżej wymienionych szczepów na wartość wskaźnika żyzności gleby (BIF) oraz plon handlowy kapusty pekińskiej. W doświadczeniu zastosowano jedenaście kombinacji, na które składał się obiekt kontrolny, gleba nawożona mineralnie, obornikiem oraz osiem obiektów, do których wprowadzono komposty wytworzone z odpadów pomidorowych lub cebulowych. Część z nich zainokulowano izolatami Trichoderma atroviride (T1) i/lub T. harzianum (T2 i T3). W trzech terminach badań pobierano próbki gleby, a następnie poddano je analizom mikrobiologicznym (ogólna liczba grzybów pleśniowych, Trichoderma sp., Fusarium sp., Alternaria sp.) oraz enzymatycznym (aktywność dehydrogenaz oraz katalazy). Ponadto po zbiorach roślin określono suchą i świeżą masę liści kapusty pekińskiej. Ogólna liczba grzybów pleśniowych oraz plon handlowy roślin w największym stopniu modyfikowane były w obiekcie z dodatkiem kompostu pomidorowego, zainokulowanego szczepem T1. Do wzrostu wartości biologicznego wskaźnika żyzności gleby (BIF) w największym stopniu przyczyniły się warianty z dodatkiem kompostów cebulowych. Stwierdzono, że właściwości fitosanitarne Trichoderma sp. wynikały ze specyfiki gatunku i tak najniższą liczebność Fusarium sp. w glebie zaobserwowano po zastosowaniu szczepu T1 wprowadzonego na nośniku w postaci kompostu cebulowego, natomiast Alternaria sp. po wprowadzeniu do gleby kompostu pomidorowego, zainokulowanego jednocześnie dwoma izolatami T1 i T2. Ponadto wykazano, że obydwa rodzaje zastosowanych kompostów okazały się dobrym nośnikiem dla izolatów Trichoderma sp.. Silniejsze namnażanie się Trichoderma sp. w glebie spowodowane było jednakże dodatkiem kompostów wytworzonym z odpadów pomidorowych.
***Słowa kluczowe:** grzyby, Trichoderma, kompost, gleba, kapusta*

1. Introduction

It is often difficult to reconcile the protection of plants by entering thousands of tonnes of chemical crop protection products into agrocenoses with the assumptions of sustainable agriculture [20]. However, the progress in crop protection which has taken place in recent years has made appropriate crop protection procedures, especially integrated protection programmes, fully in line with the concept of sustainable development [21].

Biological methods based on selected strains of microorganisms seem to be an alternative to chemical crop protection products.

The intensive development of methods of biological crop protection is possible thanks to the observation of interactions between pathogens and 'biocontrol factors', which can be found in the natural environment [28].

In recent years there has been growing interest in moulds of the *Trichoderma* genus. They are considered as the best biological control agents (BCAs), because they support the growth and development of plants and increase their resistance to pathogenic organisms [3]. It has been observed that filamentous fungi of the *Trichoderma* genus are capable of colonisation and growth when they are combined with plant roots. When the root surface has been colonised for a long time, the fungal hyphae penetrate the epidermis. In consequence, the plant may develop induced systemic resistance, which enables defence from phytopathogens [10].

Trichoderma sp. are used for the biocontrol of plant pathogens such as *Fusarium oxysporum, F. culmorum, Botrytis cinerea, Rhizoctonia solani* and *Phytophtora* [26]. The general interest in *Trichoderma* fungi is mostly caused by their properties, which make them capable of the biological control of plant pests. The fungi are characterised by rapid growth, high reproductive capacity and effective antagonistic mechanisms. Their most important role in interactions with pathogens consists in competing for nutrients and space and the production of inhibitory substances [18, 25].

The aim of this study was to assess the phytosanitary properties of *Trichoderma*strains and to prove the influence of these moulds on the biological fertility index (BIF) and the yield of napa cabbage.

2. Materials and methods

2.1. Experimental design

Two-years experiment (in 2013 and 2014) was conducted. The experiment was started in a randomised block design in plots of 9.3 m^2 (6 m x 1.55 m) belonging to a private farm in Lubosz, Commune of Kwilcz, Greater Poland Voivodeship, Poland. The experiment was located on the soil as a typical haplic luvisols formed from light loamy sands, deposited in shallow layer on light loam (Table 1).

Table 1. Chemical characteristics of soil
Tab. 1. Charakterystyka właściwości chemicznych gleby

Pure components	Value (mg kg^{-1})
N	98.0
P	35.31
K	73.87
Mg	59.12
pH_{KCl}	5.9

Source: own work / Źródło: opracowanie własne

The following three *Trichoderma* isolates were used in the experiment: *T. atroviride* (T1) and *T. harzianum* (T2 and T3). They came from the collection of strains of the Institute of Horticulture in Skierniewice, Poland. They were used for the inoculation of composts made from onion and tomato waste and then they were applied to the soil under a Chinese cabbage (*Brassica rapa* L. subsp. *Pekinensis*) Michico F1 cultivar. The composts used in the experiment were produced on a technical scale (in prisms with about 20 tonnes of input). Both the tomato waste compost and onion waste compost (mostly clusters, leaves, etc.) were mixed with wheat straw (about 10% added) and a small amount of pig manure (5%). When the thermophilic phase was over (the prism temperature was about 25°C), the composts were inoculated with *Trichoderma* strains by means of a hand sprayer (10^4cfu). One month after the inoculation of the composts they were entered into the soil. The following amounts were applied: onion waste compost – 43t ha^{-1} and tomato waste compost – 38t ha^{-1}. Apart from that, we also applied pig manure fertilisation (37 t ha^{-1}) and mineral fertilisation with nitrogen as urea (150 kg N ha^{-1}), with phosphorus as triple superphosphate (40 kg P ha^{-1}) and with potassium as potassium salt (182 kg K ha^{-1}). All organic fertilisers entered into the soil were equivalent to 170 kg N ha^{-1}. Chinese cabbage was sown by means of a manual, precision seed drill Terradonis JP-1, 20 seeds per m^2. Eleven fertiliser combinations were used in the experiment, with four replications of each combination: 1 – control sample, no fertiliser, 2 – mineral fertiliser, 3 – manure, 4 – onion waste compost, 5 – onion waste compost inoculated with strain T1, 6 – onion waste compost inoculated with strain T3, 7 – onion waste compost inoculated with strains T1 and T3, 8 – tomato waste compost, 9 – tomato waste compost inoculated with strain T1, 10 – tomato waste compost inoculated with strain T2, 11 – tomato waste compost inoculated with strains T1 and T2. Both in 2013 and 2014 soil samples necessary for microbiological and biochemical analyses were collected at three periods (ten replications), according to the Polish standard PN-ISO 10381-2:2007 [19]. Depending on the year of the research, the sample collection dates coincided with the pre-sowing phase (25-26 July) – term I, crop emergence phase (18-20 August) – term II and harvesting phase (24-29 October) – term III.

2.2. Soil microorganisms

The number of moulds in the medium was determined according to Martin [16], with rose bengal and aureomycin added. Plates were incubated for 6 days at a temperature of 25°C. The number of *Trichoderma* sp. was determined with the plate method, on a modified Martin's medium [16] 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 that *Trichoderma* sp. belonged systematically to the species of *Trichoderma harzianum* or *Trichoderma atroviride* the fungal colonies were inoculated to a PDA substrate (Sigma Aldrich). They were initially identified with a microscope and later the identification was confirmed by means of fluorescent *in situ* hybridisation (FISH) [2] with 4% PFA (paraformaldehyde), 0.5% Triton solution, alcohol series (70, 80, 96%), 70% formamide solution and two probes whose ends were marked with Cy3 marker (ACT CCC AAA CCC AAT GTG AA and ATA

CCA AAC TGT TGC CTCGG) [22]. In the experimental variants where the aforementioned *Trichoderma* sp. isolates were not applied, but the analyses revealed the presence of native *Trichoderma* sp. strains in the soil (e.g. in the control sample), only the number of *Trichoderma harzianum* and *Trichoderma atroviride* were determined. The number of *Fusarium* sp. was determined with the plate method, on a medium [14] with oxbile, chloramphenicol, streptomycin, borax and PCNB (pentachloronitrobenzene) added. The plates were incubated for 14 days at a temperature of 24°C. The number of *Alternaria* sp. was determined on a medium developed by Hong and Pryor [11] with 20% lactic acid, botran (active ingredient: dichloran) bayleton (active ingredient: triadimefon) and streptomycin added, by incubating the plates at a temperature of 24°C for 7 days. The fungal colonies were inoculated to a PDA substrate (Sigma Aldrich) and then they were identified systematically, based on mycological keys [4, 5, 7].

2.3. Soil enzymes

Biochemical analyses were performed by means of spectrophotometry. Dehydrogenases activity (DHA) was determined according to Thalmann [24] with some minor modifications. The soil (1 g) was incubated for 24 h with 2, 3, 5-triphenyltetrazolium chloride (TTC) at 30°C, pH 7.4. Triphenylformazan (TPF) was produced, extracted with 96% ethanol and measured spectrophotometrically at 485 nm. Dehydrogenases activity was expressed as μmol $TPFg^{-1}$ DM of soil $24h^{-1}$.

Catalyse activity (CAT) in the soil was determined by means of titration [13]. The soil with 0.3% H_2O_2 solution was incubated for 20 minutes and then 1.5 M H_2SO_4 was added. The resulting solution was titrated with 0.02 $MKMnO_4$. The catalyse activity was expressed as $\mu molH_2O_2$ g^{-1}D.M.of soil min^{-1}.

The dehydrogenases and catalyse activity were measured to determine the soil BIF (*Biological Index of Fertility*) according to Stefanic et al. [23].

2.4. Crops

The crops were harvested manually by collecting two central rows from the plot. The total yield and the marketable yield were separately measured. The head density was measured by counting the marketable harvested heads.

2.5. Statistical analysis

Statistical analyses were conducted by means of Statistica 12.0 software (StatSoft Inc. 2012). We used two-way analysis of variance to determine the significance of variation in the number of groups of microorganisms under analysis, depending on the soil combination and term of analysis. Moreover Least Significant Difference (LSD) tests were also used and their results are presented graphically in order to facilitate interpretation of the obtained differences at the level of the parameters under study.

3. Results and discussion

3.1. Soil microorganisms

Having averaged the results of microbiological analyses from the two years of investigations we obtained the results concerning the influence of fertilisation and the term of sample collection on the count and activity of soil microorganisms. The two-way analysis of variance, with the type of fertilisation and term of collecting soil samples from under napa cabbage plantation proved that both factors had highly significant influence on the count of the microorganisms under study and the enzymatic activity in soil.

The research showed that depending on the experimental variant, the heaviest colonisation of soil by moulds took place at the first term of investigations or at the harvest phase (the third term) – Fig. 1.

The analysis of the microbiological state of soil before fertilisation (the first term) revealed differences in the count of these microorganisms in individual soil variants (18.57-46.91 10^3cfug^{-1} DM of soil), but the differences were not statistically significant.

The proliferation of moulds decreased at the phase of plants' emergence in all soil combinations except the control variant (No. 1). It is most likely that this phenomenon was caused both by organic and mineral fertilisers entered into soil.

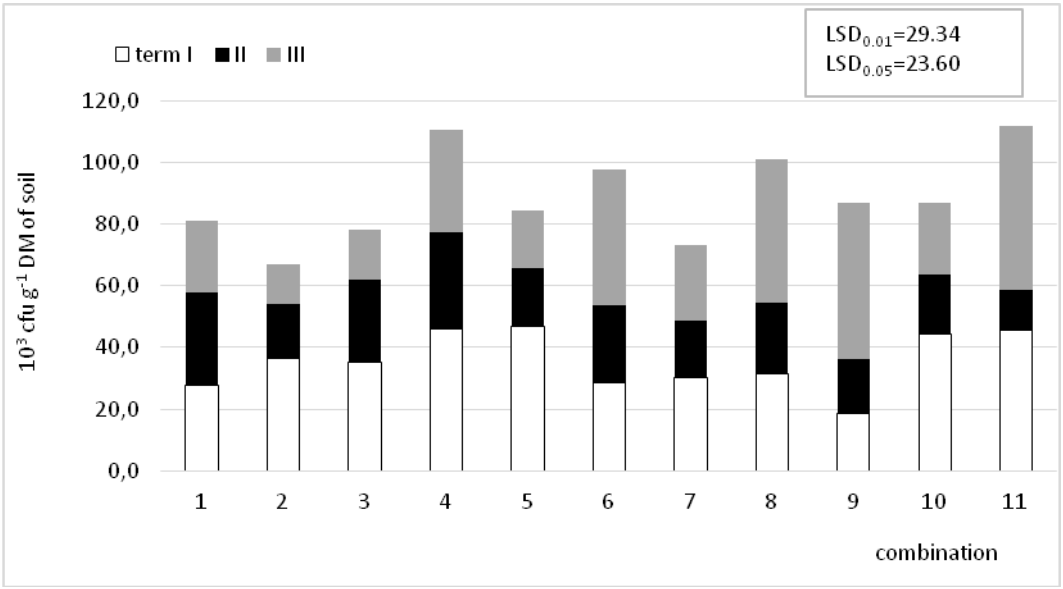
The decrease in the count of moulds in the soil fertilised with composts and manure may have been caused by the occurrence of antagonism between the autochthonous soil microflora and zymogenic microorganisms entered into soil with the composts in competing for nutrients or place of colonisation [6].

According to Hopkins and Shiel [12], the decrease in the count of fungi in the variant treated with the mineral fertiliser may have been caused by direct toxicity and changed pH due to ammonium-based fertilisers.

These observations were confirmed by the results of our study (Fig. 2), which showed that at the second term of analyses the pH value increased by 0.4 in the soil enriched with the mineral fertiliser.

The analysis of soil samples at the harvest phase (the third term) showed that there was increased proliferation of moulds only in the variants enriched with tomato or onion waste composts. However, this increase was statistically significant in two variants (No. 9 and 11). It is most likely that this phenomenon was related to the chemical composition of composts entered into the soil. According to Gniazdowska and Oracz [8], during the decomposition of plant residue in composts various substances with strong biological properties may be released.

Due to the commonness of filamentous fungi of the *Trichoderma* genus in the soil environment the soil samples collected during the research were analysed for the presence of these microorganisms. The microbiological analyses of soil at the first term of the research revealed the presence of *Trichoderma* sp. (Fig. 3) in all the experimental variants. The greatest count of these moulds was found in variant No. 2 – soil with the mineral fertiliser, whereas the smallest count was observed in the control variant (No. 1). The analysis of soil at the second term of the investigations, i.e. more than three weeks after treatment with *Trichoderma* sp. isolates, revealed that the fungi were still present in all the experimental variants, including those where no isolates were entered. It may have been caused by the presence of napa cabbage plants. According to Benìtez et al. [3], *Trichoderma* fungi are usually connected with the root zone in plants due to a similar mechanism to the one observed in mycorrhizal fungi.



Source: own work / Źródło: opracowanie własne

Fig. 1. Moulds number in soil with fertilisers addition, in three terms of analysis. Explanation: 1 – control sample, no fertilizer, 2 – mineral fertiliser, 3 – manure, 4 – onion waste compost, 5 – onion waste compost inoculated with strain T1, 6 – onion waste compost inoculated with strain T3, 7 – onion waste compost inoculated with strains T1 and T3, 8 – tomato waste compost, 9 – tomato waste compost inoculated with strain T1, 10 – tomato waste compost inoculated with strain T2, 11 – tomato waste compost inoculated with strains T1 and T2

Rys. 1. Liczebność grzybów pleśniowych w glebie z dodatkiem nawozów, w trzech terminach analiz. Objaśnienia: 1 – kontrola, bez nawożenia, 2 – nawożenie mineralne, 3 – obornik, 4 – kompost wytworzony z odpadów cebulowych, 5 – kompost wytworzony z odpadów cebulowych, zainokulowanych szczepem T1, 6 – kompost wytworzony z odpadów cebulowych, zainokulowanych szczepem T3, 7 – kompost wytworzony z odpadów cebulowych, zainokulowanych szczepami T1 i T3, 8 – kompost wytworzony z odpadów pomidorowych, 9 – kompost wytworzony z odpadów pomidorowych, zainokulowanych szczepem T1, 10 – kompost wytworzony z odpadów pomidorowych, zainokulowanych szczepem T2, 11 – kompost wytworzony z odpadów pomidorowych, zainokulowanych szczepami T1 i T2

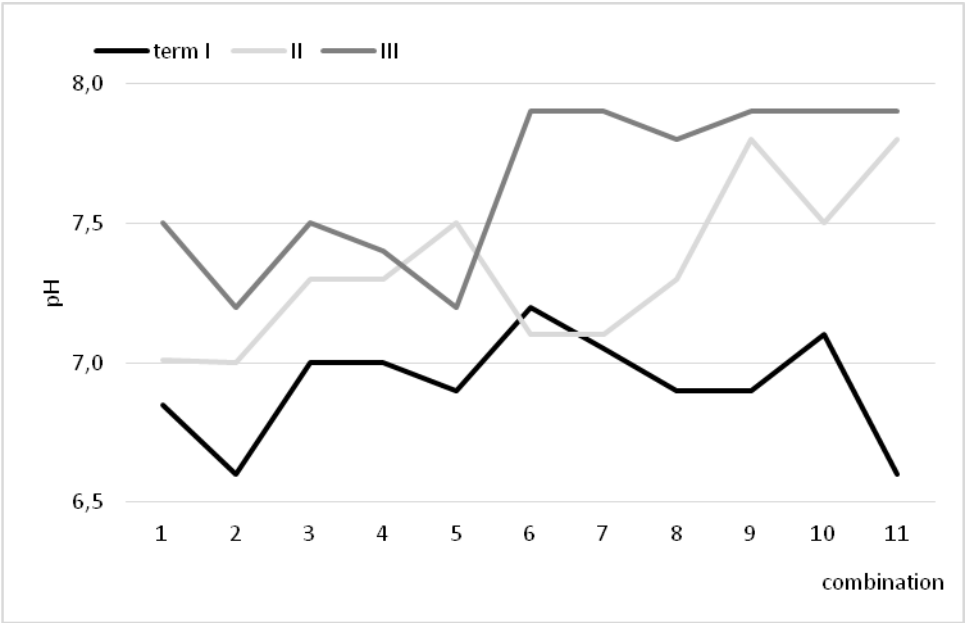


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Fig. 2. The changes of the pH value in soil combinations (Explanation as Fig. 1)

Rys. 2. Zmiany wartości pH w kombinacjach glebowych (Objaśnienia jak na rys. 1)

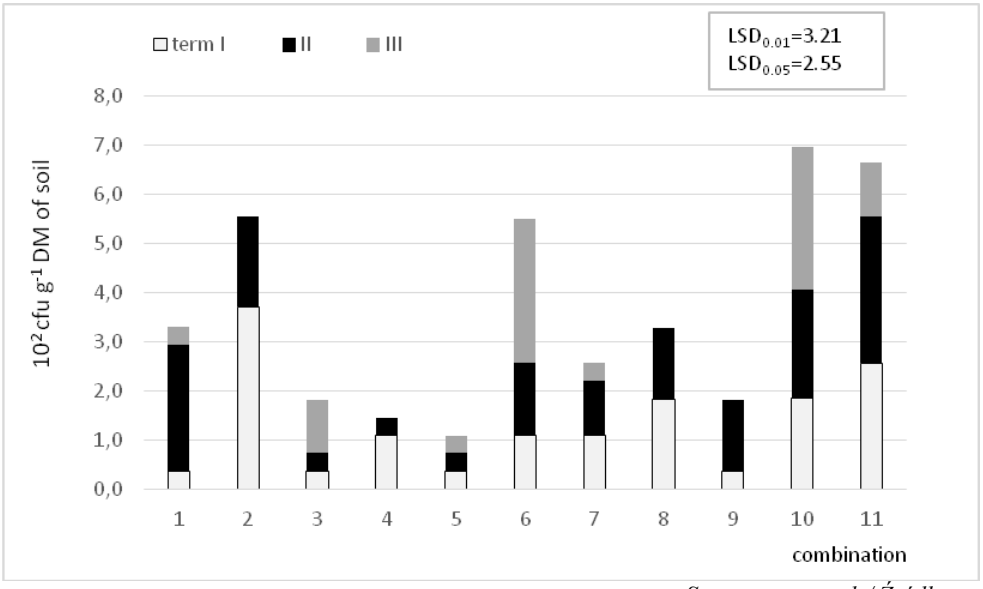

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Fig. 3. *Trichoderma* sp. number in soil with fertilisers addition, in three terms of analysis. (Explanation as Fig. 1)
Rys. 3. Trichoderrma sp. w glebie z dodatkiem nawozów, w trzech terminach analiz. (Objaśnienia jak na rys. 1)

At the third term of the investigations no *Trichoderma* sp. were found in all the experimental variants. It may have been caused by different sensitivity of *Trichoderma* sp. strains to phytoalexins, flavonoids, terpenoidsand phenols produced by plants when their roots are infected [10].

The research revealed that when composts inoculated with *Trichoderma* sp. strains were entered into the soil, the count of these microorganisms increased significantly at the third term of the research in two variants only (No.6 - soil with the onion waste compost inoculated with the T3 strain and No. 1 – soil with the tomato waste compost inoculated with the T2 strain) (Fig. 4). The results showed that when the soil was treated with the tomato waste composts (No. 9-11),the growth and development of *Trichoderma* sp. were more dynamic than after treatment with the onion waste composts (No. 5-7).

The analysis of the sanitary state of soil at the first term of the research revealed the presence of *Fusarium* sp. (Fig. 5) and *Alternaria* sp. (Fig. 6) fungi n all the soil variants. The variants prepared for fertilisation with composts were the most contaminated (No. 4, 10 and 11).

At the second term of the research, which corresponded to the phase of vegetative growth of plants in most of the experimental combinations, the proliferation of *Fusarium* sp. increased. The increase was particularly noticeable in the soil fertilised organically with manure as well as onion and tomato waste composts.

There were analogical results of the studies by Wolna-Maruwka et al. [29] and Wolna-Maruwka et al. [28], who analysed the count of *Fusarium* sp. in the soil under a beet-root plantation. The authors observed more dynamic proliferation of these microorganisms at the phase of plants' emergence. It is most likely that the phenomenon was caused by the presence of plants and their root secretions, which caused physicochemical changes in the soil. According to Lee [15], the increase in the count of moulds in soil may also be the result of agrotechnical procedures applied during cultivation, e.g. organic fertilisation.

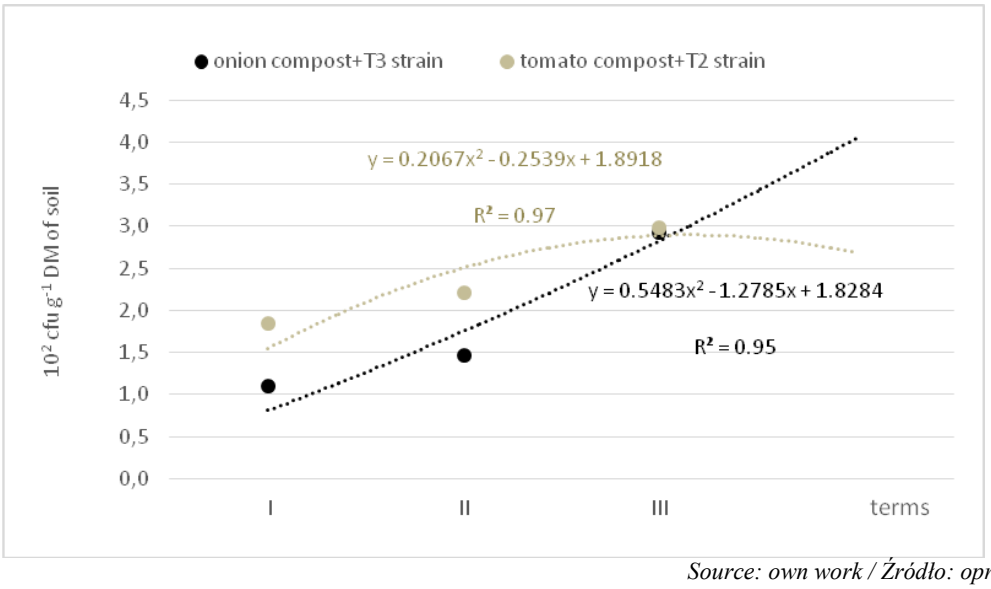

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Fig. 4. Impact of composts fertilization on the number of *Trichoderma* sp.
Rys. 4. Wpływ nawożenia kompostami na liczebność Trichoderma sp.

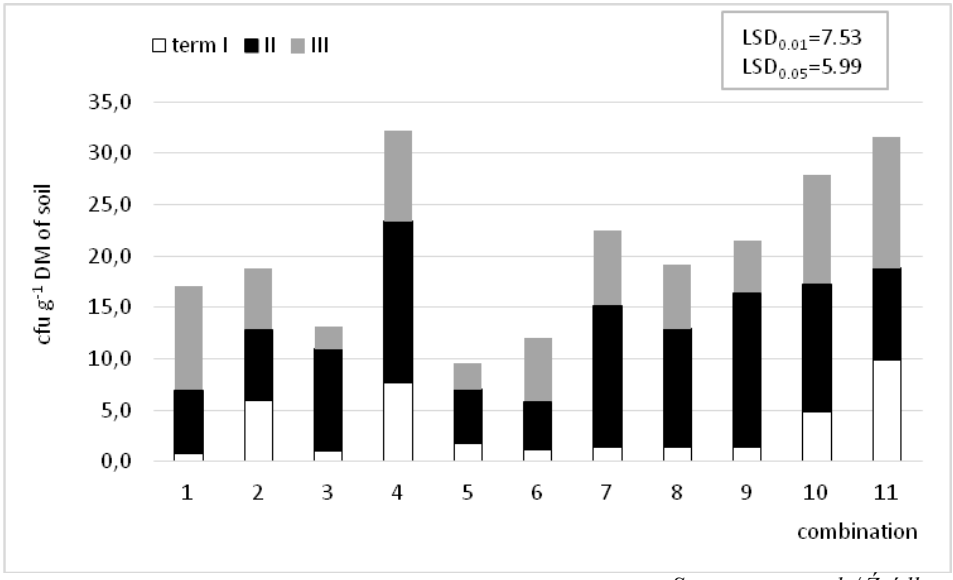

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Fig. 5. *Fusarium* sp. number in soil with fertilisers addition, in three terms of analysis. (Explanation as Fig. 1)
Rys. 5. Fusarium sp. w glebie z dodatkiem nawozów, w trzech terminach analiz. (Objaśnienia jak na rys. 1)

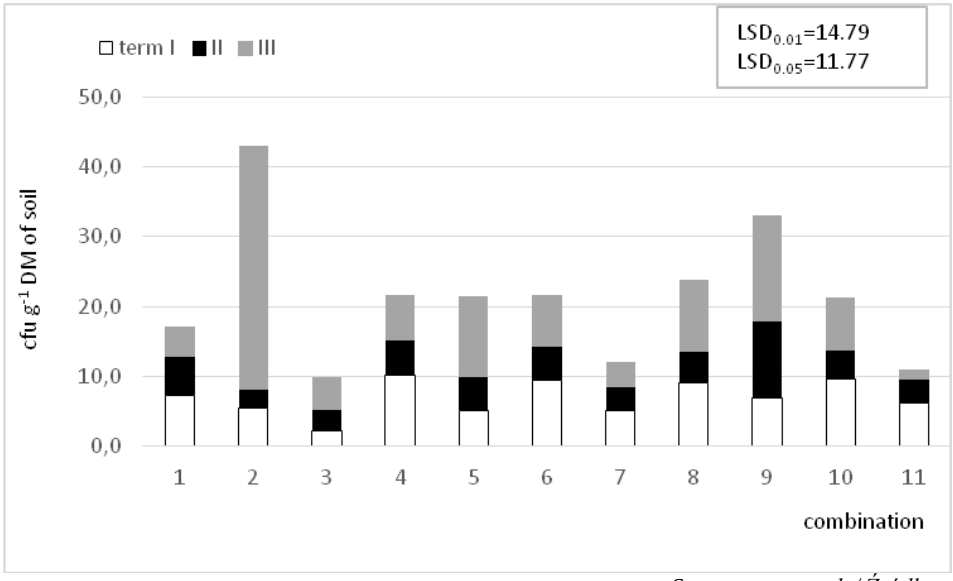

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Fig. 6. *Alternaria* sp. number in soil with fertilisers addition, in three terms of analysis. (Explanation as Fig. 1)
Rys. 6. Alternaria sp. w glebie z dodatkiem nawozów, w trzech terminach analiz. (Objaśnienia jak rys. 1)

There was a statistically insignificant decrease in the count of *Alternaria* sp.(Fig. 6) in most of the experimental variants at the phase of plants' emergence, but the count increased again at the harvest phase (the third term). The intensity of proliferation of *Fusarium* moulds at the third term was diversified and it depended on the type of experimental combination. The comparison of the sanitary state of soil at the first and third term of the research showed that the most intense proliferation of *Fusarium* sp. took place in the control sample, whereas the least intense proliferation was observed in the variant treated with the mineral fertiliser. Apart from that, fertilisation with the onion waste compost inoculated with the T1 strain inhibited the proliferation of these microorganisms.

The growth and development of *Alternaria* sp. were most inhibited in the soil fertilised with the tomato waste compost inoculated with the T1 and T2 strains. According to Witkowska and Mai [27], the phytosanitary properties of *Trichoderma* sp. result from the production of volatile and non-volatile compounds, where the effect of non-volatile secondary metabolites (enzymes and antibiotics) is thought to be stronger.

3.2. Soil enzymes

The biochemical analyses of soil showed that at the first term of the research the biological fertility index (BFI) reached the highest values in most of the experimental variants except those treated with the onion waste compost (No. 4-7) – Fig. 7.

At the consecutive terms of investigations the enzymatic activity of soil fluctuated, depending on the experimental variant. The comparison of enzymatic activity values between the first and third term of investigations showed that the smallest increase in the activity was noted in the soil enriched with the tomato waste compost without inoculation (No. 8) and in the variant treated with the mineral fertiliser (No. 2).

According to Acosta-Martinez and Tabatabai [1], despite the fact that mineral fertilisation has positive effect on the physicochemical properties of soil, its enzymatic activity may be reduced due to the presence of easily assimilable forms of mineral compounds.

The results of our research also showed that at the three terms of the investigations the highest enzymatic activity was observed in the combination with the onion waste compost inoculated with the T3 strain.

Our observations were confirmed by the study conducted by Meen et al. [18], who proved that the dehydrogenases activity in the soil fertilised organically (with manure) was higher than in the soil treated with the mineral fertiliser.

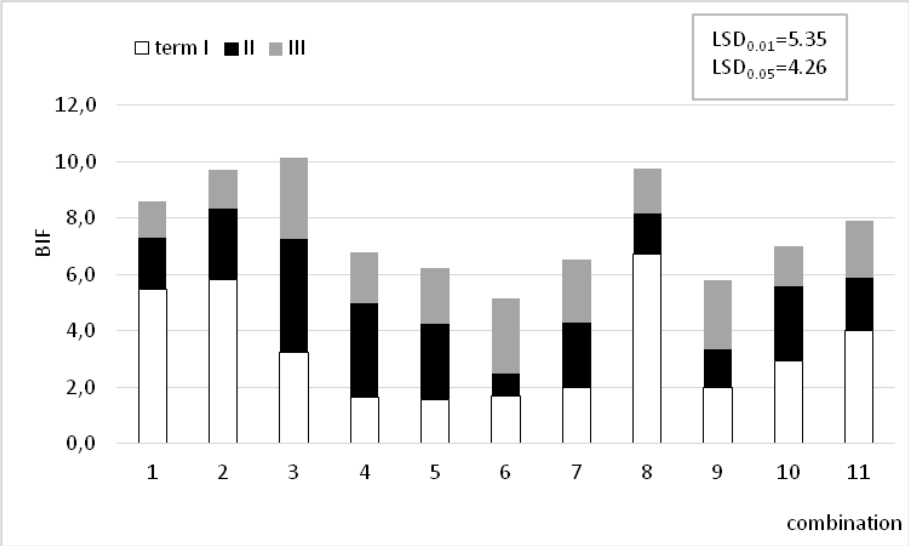
3.3. Crop plants

The analysis of the commercial yield of napa cabbage (Table 2) showed that in the control variant, where no fertiliser was applied, the total yield of the fresh weight of napa cabbage amounted to 54.3 t ha^{-1}. The total yield of the fresh weight of napa cabbage was significantly greater than in the control variant when manure (79.9 t ha^{-1}), the onion waste compost with the T1 strain (75.0 t ha^{-1}) and the same compost without microbial additives (69.6 t ha^{-1}) were applied. The study by Haque et al. [9] showed that the efficient use of *Trichoderma*-enriched compost may increase the yield, reduce the use of nitrogen fertilisers, reduce soil- borne pathogens and improve the soil health. Other fertilisers did not cause a statistically significant increase in the yield in comparison with the control variant.

The manure fertiliser resulted in the highest commercial yield of napa cabbage (57.3 t ha^{-1}). The other fertilisers gave a significantly lower yield. Only when the mineral fertiliser, the onion waste compost and the same compost with the T1 strain were applied, the yield did not differ significantly from the highest yield. The yield of cabbage in the variants fertilised with the onion waste compost without additives and the same compost with the T3 strain did not differ from the yield in the control variant without fertilisation.

The number of commercial heads of cabbage in the control variant amounted to 5.7 pieces per m^2. Only fertilisation with the tomato waste compost with the T1 strain resulted in a significantly greater number of heads than in the control variant without fertilisation.

The average weight of heads in the control variant was 0.61 kg. The mineral fertiliser, manure and the tomato waste compost with the T1 and T2 strains caused a significant increase in the weight of an individual head of napa cabbage.



Source: own work / Źródło: opracowanie własne

Fig. 7. BIF index values in soil with fertilisers addition, in three terms of analysis. (Explanation as Fig. 1)
Rys. 7. Wartości wskaźnika BIF w glebie z dodatkiem nawozów, w trzech terminach analiz. (Objaśnienia jak na rys. 1)

Table 2. Total and commercial yield, number and weight of cabbage head
Tab. 2. Plon ogólny i plon handlowy, liczba główek i masa główki kapusty pekińskiej

Combination	Total yield (t ha^{-1})	Commercial yield (t ha^{-1})	Number of cabbage heads (number m^{-2})	Average weight of the head (kg)
1	54.3	35.5	5.7	0.61
2	65.3	48.1	4.3	1.12
3	79.9	57.3	4.6	1.34
4	64.0	44.0	5.8	0.75
5	66.7	46.4	5.8	0.78
6	58.6	42.0	4.7	0.90
7	65.9	46.6	5.4	0.85
8	69.6	48.5	5.4	0.93
9	75.0	53.9	7.0	0.75
10	64.2	46.1	5.4	0.85
11	62.8	44.7	4.4	0.99
$LSD_{0.05}$	16.0	9.6	1.9	0.33

Explanation as Fig. 1.

Source: own work / Źródło: opracowanie własne

4. Conclusions

1. The count of moulds was most strongly modified by treating the soil with the tomato waste compost inoculated with the T1 strain and the onion waste compost with the T3 strain.
2. The variants with the onion waste composts caused the greatest increase in the biological fertility index (BIF).
3. The smallest count of *Fusarium* sp. in soil was observed after fertilisation with the onion waste compost with the T1 strain, whereas the smallest count of *Alternaria* sp. was noted after treatment with the tomato waste compost, which was simultaneously inoculated with two isolates, i.e. T1 and T2.
4. The greatest count of moulds of the *Trichoderma* sp. genus was noted in the soil enriched with the onion waste compost with the T3 strain and in the variant treated with the tomato waste compost inoculated with the T2 *Trichoderma* strain.
5. The highest commercial yield of napa cabbage resulted from the application of manure and the tomato waste compost inoculated with the T1strain.
6. The study showed that vegetable waste could be used as a carrier for entering *Trichoderma* sp. isolates into soil. They increased the commercial yield of napa cabbage and limited the development of selected plant pathogens. However, it is important to select an adequate *Trichoderma* sp. strain.

5. References

[1] Acosta-Martinez V., Tabatabai M.A.: Enzyme activities in a limed agricultural soil. Biology and fertility of soils, 2000, 31(1), 85-91.
[2] Amann R.I., Krumholz L., Stahl D.A.: Fluorescent oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. Journal of Bacteriology, 1990, 172 (2), 762-770.
[3] Benítez T., Rincón A. M., Limón M. C., Codón A.C.: Biocontrol mechanisms of *Trichoderma* strains. International microbiology, 2004, 7(4), 249-260.
[4] Domsch K.H., Gams W., Anderson T.H.: Compendium of soil fungi, Volume 1. Academic Press, San Francisco, 1980, 406.
[5] Ellis M.B.: More Dematiaceous hyphomycetes. Commonwealth Mycological Institute. Surrey, Kew, England, 1976, 507.
[6] Garbeva P., Van Veen J.A., Van Elsas J.D.: Microbial diversity in soil: Selection of microbial populations by Plant and Soil Type and Implications for Disease Suppressiveness. Annual Review Phytopathology, 2004, 42, 243-270.
[7] Gerlach W., Nirenberg H. J.: The genus *Fusarium* - a pictorial atlas. Mitteilungen aus der Biologischen Bundesanstalt fur Land-und Forstwirtschaft Berlin-Dahlem, 1982.
[8] Gniazdowska A., Oracz K., Bogatek R.: Allelopatia-nowe interpretacje oddziaływań między roślinami. Kosmos, 2004, 53(2), 207-217.
[9] Haque M.M., Ilias, G.N.M., Molla A.H.: Impact of *Trichoderma*-enriched Biofertilizer on the Growth and Yield of Mustard (*Brassica rapa* L.) and Tomato (Solanumlycopersicon Mill.). The Agriculturists, 2012, 10(2), 109-119.
[10] Harman G.E., Howell C.R., Viterbo A., Chet I., Lorito M.: *Trichoderma* species – opportunistic, avirulent plant symbionts. Nature reviews microbiology, 2004, 2(1), 43-56.
[11] Hong B., Pryor M.: Development of selective media for the isolation and enumeration of Alternaria species from soil and plant debris. Canadian Journal of Microbiology, 2004, 50, 461-468.
[12] Hopkins D.W., Shiel R.S.: Size and activity of soil microbial communities in long-term experimental grassland plots treated with manure and inorganic fertilizers. Biology and Fertility of Soils, 1996, 22, 66–70.
[13] Johnson J.I., Temple K.L.: Some variables affecting the measurements of catalase activity in soil. Soil Science Society of America Proceedings, 1964, 28, 207-209.
[14] Komada H.: Development of a selective medium for quantitative isolation of *Fusariumoxysporum* from natural soil. Reviev of Plant Protection Research, 1975, 8, 114-124.
[15] Lee J.: Effect of application methods of organic fertilizer on growth, soil chemical properties and microbial densities in organic bulb onion production. Scientia Horticulturae, 2010, 124 , 299-305.
[16] Martin J.P.: Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Science, 1950, 69, 215-232.
[17] Meena V.S., Maurya B.R., Meena R.S., Meena S.K, Singh N.P., Malik V.K., Kumar V., Jat L.K.: Microbial dynamics as influenced by concentrate manure and inorganic fertilizer in alluvium soil of Varanasi. India. African Journal Microbiology Research, 2014, 8(3), 257-263.
[18] Mukherjee P.K., Horwitz B.A., Kenerley C.M.: Secondary metabolism in *Trichoderma* – A genomic perspective. Microbiology, 2012, 158, 35-45.
[19] PN-ISO 10381-2:2007 Soil quality – Collecting Samples – Part 2: The Rules of Collection Techniques.
[20] Polish Crop Protection Products Regulation of 8 March 2013, Official Journal, Pos. 455, 2013.
[21] Pruszyński S., Wolny S.: Przewodnik Dobrej Praktyki Ochrony Roślin. Instytut Ochrony Roślin. Poznań 2007..
[22] Siddiquee S., Yusof N.A., Salleh A.B., Tan S.G., Bakar F.A., Heng L.Y.: DNA hybridization based on *Trichoderma harzianum* gene probe immobilization on self-assembled monolayers on a modified gold electrode. Sensors and Actuators B: Chemical, 2010, 147, 198-205.
[23] Stefanic F., Ellade G., Chirnageanu J.: Researches concerning a biological index of soil fertility. [in:] Nemes M.P., Kiss S., Papacostea P., Stefanic C., Rusan M., ed. Proceeding of the Fifth Symposium of Soil Biology. Bucharest, Romania. Romanian National Society of Soil Science, 1984, 35-45.
[24] Thalmann A.: Zur Methodik der Bestimmung der Dehydrogenase Aktivität in Boden mittels Triphenyltetrazoliumchlorid (TTC). Landwirtschaftliche Forschung, 1968, 21, 249-258.
[25] Toghueo R.M.K., Eke P., Zabalgogeazcoa I., Rodríguez Vázquez De Aldana B., Nana L. W., Boyom F.F.: Biocontrol and growth enhancement potential of two endophytic *Trichoderma* spp. from *Terminalia catappa* against the causative agent of Common Bean Root Rot (*Fusarium solani*). Biological Control, 2016, 96, 8-20.
[26] Vinale F., Sivasithamparam K., Ghisalberti E.L., Marra R., Woo S.L., Lorito M.: *Trichoderma*–plant–pathogen interactions. Soil Biology and Biochemistry, 2008, 40(1), 1-10.
[27] Witkowska D., Maj A.: Production of lytic enzymes by *Trichoderma* spp. and their effect on the growth of phytopathogenic fungi. Folia Microbiologica, 2002, 47, 3, 79-282.
[28] Wolna-Maruwka A., Piechota T., Dach J., Szczech M., Szczerbal I., Niewiadomska A., Budka A., Gaj R.: The influence of *Trichoderma* on the phytosanitary status of soil and yield of red beets (*Beta vulgaris* L. subsp. *vulgaris*). Polish Journal Environmental Studies, 2017, 26(2), 847-859.
[29] Wolna-Maruwka A., Piechota T., Kosicka-Dziechciarek D., Szczech M., Niewiadomska A., Dach J.: A mycological analysis of soil fertilised with vegetable waste composts under a radish (*Raphanus sativus*) plantation. Journal of Research and Applications in Agricultural Engineering, 2016, 61(4), 223-229.

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".