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A MYCOLOGICAL ANALYSIS OF SOIL FERTILISED WITH VEGETABLE WASTE COMPOSTS UNDER A RADISH (*RAPHANUS SATIVUS*) PLANTATION

Summary

Conducted study concerns the influence of fertilisation (manure, onion and tomato compost) and three strains of Trichoderma isolated from soils on the total number of moulds, including plant pathogens of the Fusarium and Alternaria and the yield of radish. The research proved that the number of the microorganisms were statistically significantly modified by the type of combination and the term of analyses. The number of moulds was the most strongly influenced by the addition of manure. On the other hand, the number of the Trichoderma was the highest in the soil enriched with tomato waste composts. The study did not prove any antagonistic effect of Trichoderma sp. isolates on the proliferation of Alternaria sp. The growth and development of Fusarium sp. was inhibited by onion compost with addition of strain Trichoderma - T3. The yield of radish was the highest in the soil fertilised with the manure and tomato composts with Trichoderma sp.- T1.
***Key words**: moulds, pathogen, compost, soil, radish*

ANALIZA MIKOLOGICZNA GLEBY NAWOŻONEJ KOMPOSTAMI Z OPADÓW WARZYWNYCH POD UPRAWĄ RZODKWI (*RAPHANUS SATIVUS*)

Streszczenie

Przeprowadzono badania, których celem było wykazanie wpływu nawożenia mineralnego i organicznego (ornikiem, kompostem cebulowym i pomidorowym) oraz trzech szczepów Trichoderma sp., wyizolowanych z gleby na ogólną liczebność grzybów pleśniowych, w tym patogenów roślinnych z rodzaju Fusarium i Alternaria, jak również plon rzodkwi.
Wykazano, że liczebność analizowanych mikroorganizmów istotnie statystycznie modyfikowane były rodzajem kombinacji doświadczalnej oraz terminem analiz. Na liczbę grzybów pleśniowych najsilniej wpływał dodatek obornika. Z kolei namnażanie Trichoderma najsilniej stymulowały komposty wytworzone z odpadów pomidorowych. Nie wykazano inhibicyjnego wpływu zastosowanych izolatów Trichoderma sp. na namnażanie się Alternaria sp.. Ograniczenie rozwoju Fusarium sp. odnotowano w kombinacji z dodatkiem kompostu cebulowego, zainokulowanego szczepem T3. Plon korzeni i liści rzodkwi osiągnął najwyższe wartości w obiekcie nawożonym obornikiem oraz kompostem wytworzonym z odpadów pomidorowych z dodatkiem szczepu T1.
***Słowa kluczowe**: pleśnie, patogeny, kompost, gleba, rzodkiew*

1. Introduction

Rapid development of agriculture and legal restrictions concerning the use of chemical plant protection products cause improvement in studies on biological methods of plant protection, which are based on natural phenomena [1]. On 1 January 2014 the European Union introduced obligatory integrated crop protection. In Poland it was introduced as a Regulation of the Minister of Agriculture and Rural Development [2]. According to the rules of integrated crop protection specified in Annex III of Directive 2009/128/EC, 'Sustainable biological, physical and other non-chemical methods must be preferred to chemical methods if they provide satisfactory pest control' [3].

According to Sigh et al. [4], the use of chemical plant protection products may destroy natural biodiversity of soil and reduce its fertility. In modern plant growing technology we can observe an increasing frequency of application of formulations based on microorganisms isolated from natural environments, such as filamentous fungi of the *Trichoderma* genus [5]. These fungi produce numerous antibiotics and enzymes and exhibit parasitic effect on plant pathogens [6]. *Trichoderma* sp. have been globally regarded as one of the most outstanding fungal species exhibiting antagonistic effect on pathogenic organisms, such as: *Fusarium* sp., *Alternaria* sp., *Rhizoctonia* sp., *Phythium* sp. [7, 8].

The phytosanitary properties of *Trichoderma* sp. and their capacity to promote the growth of plants cause formulations based on these fungi to be an alternative to chemical plant protection products.

The aim of the study was to analyse the influence of mineral and organic fertilisation on the count of moulds, including *Trichoderma* sp., *Alternaria* sp. and *Fusarium* sp., and the influence on the yield of radish. Apart from that, the aim of the study was to prove the type of interaction between the applied *Trichoderma* sp. isolates and pH value.

2. Materials and methods

2.1. Experimental design

Two-year experiment (in 2013 and 2014) was conducted. The experiment was started in a randomised block design in plots of 9.3m^2 (6m x 1.55m) 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 radish (*Raphanus sativus L. var. Longipinnatus Bailey)*, Japana 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 (10^4 cfu). One month after the inoculation of the composts they were entered into the soil. The following amounts were applied: onion waste compost – 43 t ha^{-1} and tomato waste compost – 38 t ha^{-1}. Apart from that, we also applied pig manure fertilisation (37 t ha^{-1}) and mineral fertilisation with nitrogen as urea (90 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}.

Radish seeds were 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 [9]. Depending on the year of the research, the sample collection dates coincided with the pre-sowing phase (22-26 July) – term I, crop emergence phase (17-18 August) – term II and harvesting phase (10-11 October) – term III.

2.2. Soil microorganisms

The number of moulds in the medium was determined according to Martin [10], 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 [10] 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) [11] 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) [12].

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 [13] 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 [14] 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 [15-17].

2.3. Crops

The crops were harvested manually by collecting two central rows from the plot. The yield of aerial parts and the yield of roots were separately measured. The crop density was measured by counting the plants harvested.

2.4. 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.

Pearson's linear correlation coefficient was used to determine the correlation between the number of *Trichoderma* sp. and pH value.

3. Results and discussion

Having averaged the results of microbiological analyses conducted during two years of the study, we obtained results concerning the influence of fertilisation and the term of collecting samples on the number of moulds (Figure 1). The quantitative analysis of moulds proved that the most intensive colonisation of soil with these microorganisms took place at the first (before sowing) and third (harvest) term of analyses and it depended on the type of the experimental object. During the experiment the greatest number of these microorganisms was observed in the soil fertilised with manure (combination 3), whereas the lowest count was observed in the object where mineral fertilisation had been applied (combination 2). These observations are confirmed by the study by Martyniuk et al. [18], who found that liquid manure fertilisation increased the count of microorganisms and their respiratory activity.

According to Bielińska & Mocek [19] and Barabasz & Vořišek [20], the type of fertiliser is a significant factor modifying the number of soil microorganisms and their activity.

In most cases onion or tomato waste composts reduced the count of moulds by 23-26% on average. This situation may have been caused by antagonisms concerning nutrients or place of colonisation between autochthonous soil microflora and zymogenous organisms entered into the soil with composts. It may also have been caused by products of decomposition of organic matter in composts, which exhibited properties inhibiting the growth of moulds.

The analysis of the number of *Trichoderma* sp. in soil showed that both the type of experimental combination and the term of sample collection had significant influence on the proliferation of these microorganisms (Figure 2).

The greatest number of these microorganisms was observed in the experimental objects at the first term of analyses (before sowing). This means that *Trichoderma* sp. strains were part of the autochthonous microflora naturally colonising the soil.

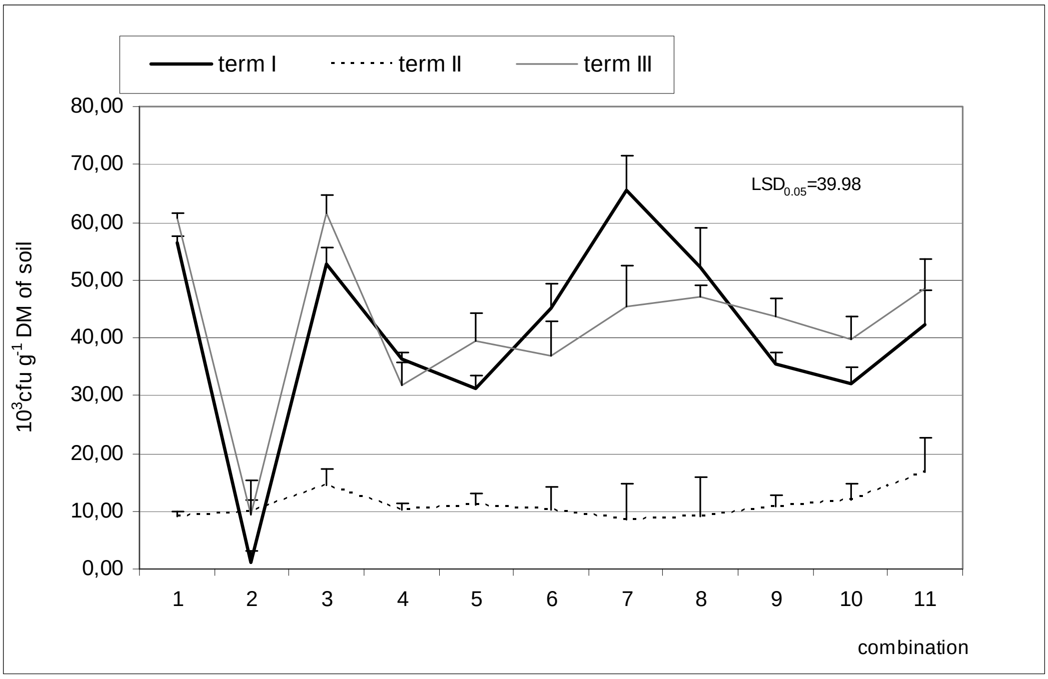
At the stage of plant emergence (the second term) in most soil combinations the number of these microorganisms decreased. Increased proliferation of the microorganisms could be observed at the harvest time (the third term). It is most likely that this effect was caused by the presence of radish plants, whose root secretions initially inhibited the growth and development of *Trichoderma* sp. According to Walker et al. [21], the qualitative and quantitative composition of root secretions depends both on the species of a plant and its stage of development. Root secretions may both stimulate and inhibit the proliferation of soil microorganisms.

Variation in the soil pH may have been another factor affecting variation in the proliferation of *Trichoderma* sp. in the objects under analysis.

At the first term of analyses, apart from the soil where mineral fertilisation had been applied, the soil pH reached the lowest value (Figure 3) and the greatest count of the moulds under study was observed. This tendency was particularly noticeable in object No. 5 (soil enriched with onion waste compost and isolate T1).

As results from the data available in literature, fungi of the *Trichoderma* genus develop best in a substrate with an acidic pH (2.0-6.5), but the pH range of 5.0-5.5 is considered to optimal for their growth and development [22].

Apart from that, as results from the study by Galus-Barchan and Paśmionka [23], the count of soil microorganisms is also influenced by soil humidity, the presence of nutrients, air supply, pH and even the soil structure.

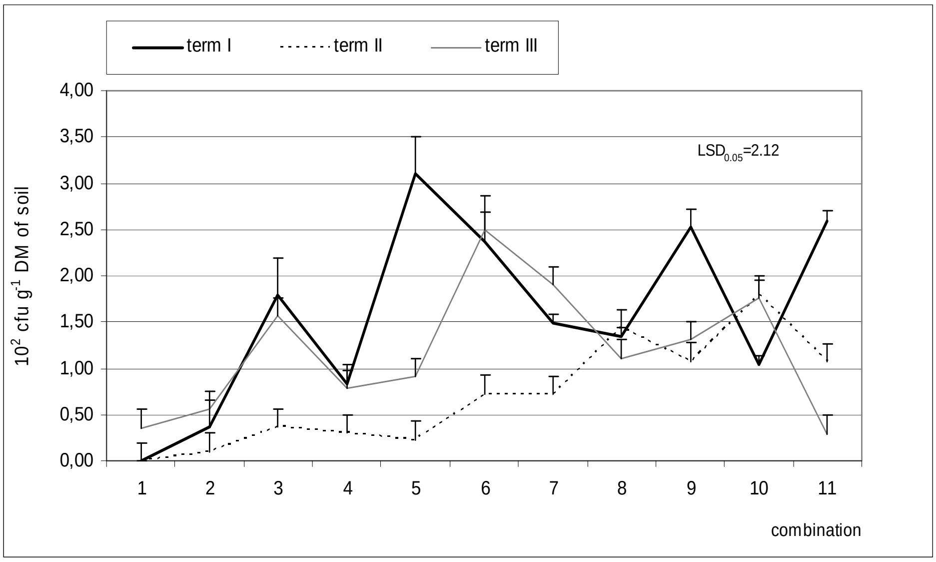


Explanation: Means followed by the same letters do not differ significantly at *p*=0.05; Combination: 1 – control sample, no fertilizer, 2 – mineral fertilizer, 3 – manure, 4 – onion waste compost, 5 – onion waste compost inoculated with strain T1, 6 – onion waste compost inoculated with strain T3, 7 – 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; Terms: I - the pre-sowing phase, II - crop emergence phase, III - harvesting phase.

Source: own work / *Źródło:* opracowanie własne

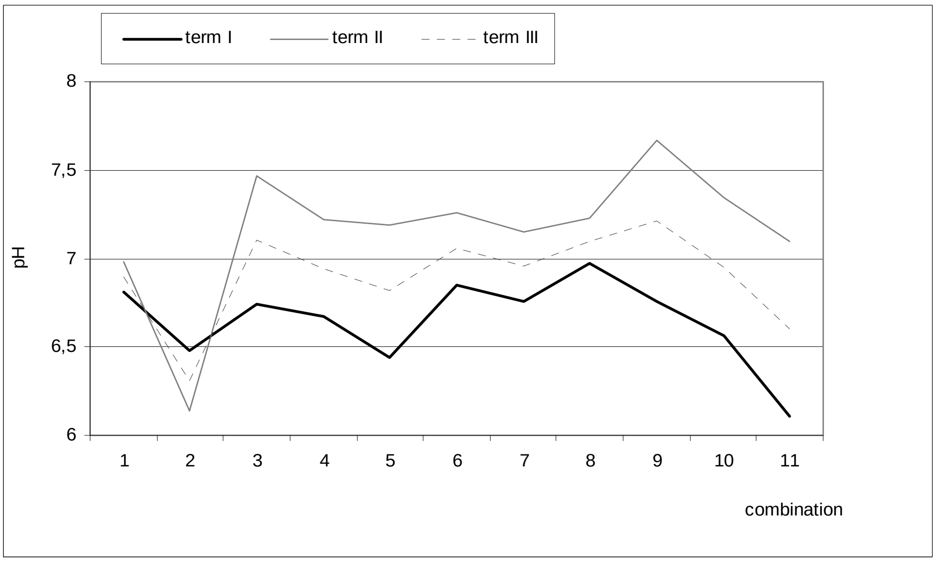
Fig. 1. Moluds number in soil with fertilisers addition, in three terms of analysis

Rys. 1. Liczebność grzybów pleśniowych w glebie z dodatkiem nawozów, w trzech terminach analiz



Source: own work / *Źródło:* opracowanie własne

Fig. 2. *Trichoderma* sp. number in soil with fertilizers addition, in three terms of analysis
Rys. 2. Trichoderma sp. *w glebie z dodatkiem nawozów, w trzech terminach analiz*



Source: own work / *Źródło:* opracowanie własne

Fig. 3. The changes of the pH value in the soil
Rys. 3. Zmiany wartości pH w glebie

During the experiment the most statistically significant influence of soil pH on the number of *Trichoderma* sp. was observed in object No. 5. This observation was confirmed by statistical analysis, where Pearson's linear correlation coefficient was calculated (Table 2).

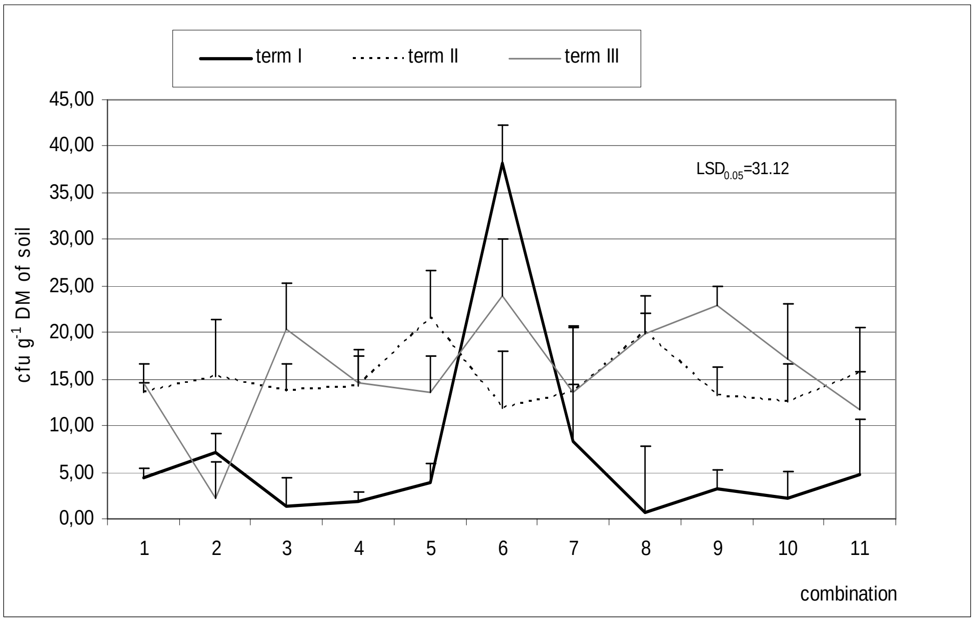
Our research findings showed that both types of composts caused a comparable increase in the count of *Trichoderma* sp. in the soil. The greatest number of *Trichoderma* sp. was observed in the soil where tomato compost inoculated with strain T2 (object No. 10) was applied (object No. 10). The second greatest count of *Trichoderma* sp. was found in the soil enriched with onion compost and isolates T1 and T3 (object No. 7).

In order to confirm the phytosanitary properties of *Trichoderma* sp. the count of common soil pathogenic fungi of the *Alternaria* and *Fusarium* genera was analysed (Figure 4, 5).

Table 2. Yield of roots and leafs of radish as affected by fertilization, mean from 2013-2014 (Mg ha-1)
Tab. 2. Plon świeżej masy korzeni i liści rzodkwi korzeniowej, w zależności od nawożenia, średnio za lata 2013–2014 [Mg ha^{-1}]

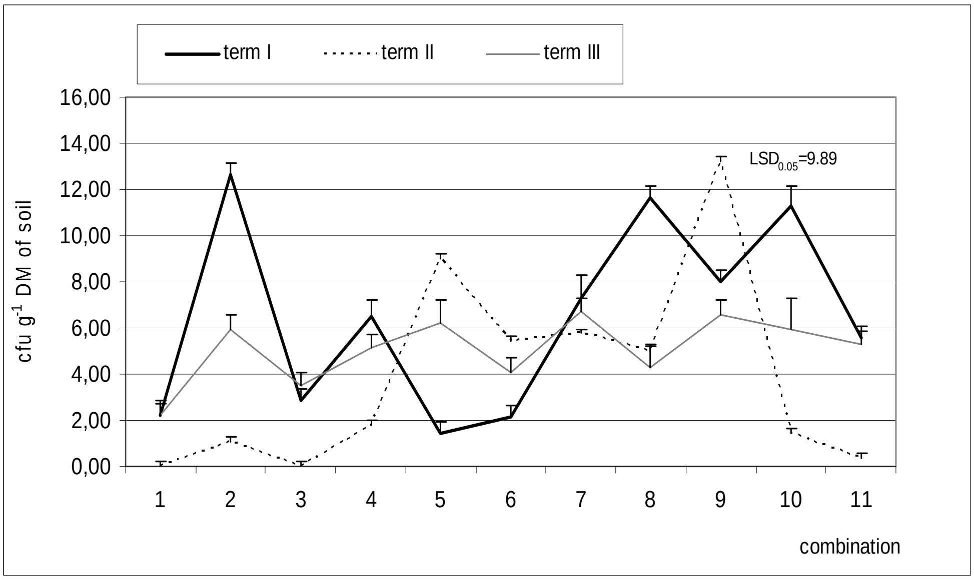
Combination	Yield		
	Roots	Leafs	Total
1	28.9	11.6	40.5
2	37.8	21.6	59.4
3	44.7	19.3	63.9
4	27.1	11.4	38.4
5	33.2	11.5	44.7
6	33.2	10.8	44.0
7	34.4	11.5	45.9
8	33.8	12.6	46.4
9	45.2	16.4	61.6
10	33.3	11.4	44.7
11	37.4	12.7	50.1
$LSD_{0.05}$	12.0	6.7	17.3

Source: own work / *Źródło:* opracowanie własne



Source: own work / *Źródło:* opracowanie własne

Fig. 4. *Fusarium* sp. number in soil with fertilizers addition, in three terms of analysis
Rys. 4. Fusarium sp. *w glebie z dodatkiem nawozów, w trzech terminach analiz*



Source: own work / *Źródło:* opracowanie własne

Fig. 5. *Alternaria* sp. number in soil with fertilisers addition, in three terms of analysis
Rys. 5. Alternaria sp. *w glebie z dodatkiem nawozów, w trzech terminach analiz*

The sanitary analysis of soil at the first term of investigations showed the presence of these pathogenic fungi in the experimental combinations. At the stage of plant emergence (the second term) the count of *Fusarium* sp. increased in all soil objects, except combination No. 6, where the soil was enriched with onion compost inoculated with isolate T3. In most objects the increased proliferation of these microorganisms continued until the third term. It may have been caused by the presence of allelopathic compounds secreted by plants' roots [24] or by diversified reaction of *Fusarium* sp. to the fertilisers applied in the experiment.

The count of fungi of the *Alternaria* sp. genus fluctuated at the second and third term of investigations, depending on the type of experimental combination.

As results from an overview of literature, there are different opinions concerning the influence of fertilisation on the mycological state of soil. The study by Awad and Fawzy [25] proves the promoting influence of organic fertilisation (sewage sludge) on the growth and development of moulds in soil. On the other hand, Hoitink and Boehm [26] report that organic fertilisation inhibits the development of some soil fungi, including pathogens from the *Pythium*, *Phytophthora* and *Fusarium* genera.

Apart from that, the sanitary analysis of soil showed that only the onion compost inoculated with strain T3 caused a statistically significant decrease in the count of *Fusarium* sp. in the soil. The greatest decrease in the count of *Alternaria* sp. (the third term of investigations) was noted in the soil fertilised with the tomato waste compost without *Trichoderma* sp. (object No. 8), in the soil fertilised with the compost inoculated with strain T2 (object No. 10) and in the soil where mineral fertilisation had been applied (object No. 2).

As results from the data in Table 3, the type of fertilisation is a factor that modifies not only the number of soil microorganisms but also and above all the yield of crops. Our research findings proved that 28.9 $Mg \cdot ha^{-1}$ of radish roots were harvested from the unfertilised control plot. Of all fertilisers used in the experiment an increase in the yield was proved only in the combination with manure and the one with the tomato waste compost inoculated with isolate T1. According to Haque et al. [27], efficient use of *Trichoderma*-enriched compost may increase yield, reduce the uses of N fertilisers, reduce soil borne pathogens and improve soil health.

Table 3. Correlation coefficients between the *Trichoderma* sp. Number and pH value

Tab. 3. Współczynnik korelacji pomiędzy liczebnością Trichoderma sp. a wartością pH

Combinations	Correlation coefficients
1	-0,50
2	0,56
3	-0,92
4	-0,90
5	-0,95*
6	-0,83
7	-0,64
8	0,25
9	-0,93*
10	0,88
11	-0,65

*correlation coefficient significant at significance level $\alpha = 0.05$

Source: own work / *Źródło:* opracowanie własne

It was also observed an unproved tendency for yield to increase when compost was inoculated with a mixture of strains T1 and T3 and after mineral fertilisation. It was observed a similar dependence as regards the yield of radish leaves – a significant increase in the yield after the application of manure and mineral fertiliser. There was also an unproved increasing tendency after the application of the tomato waste compost inoculated with isolate T1.

Manure, mineral fertilisation and the tomato waste compost inoculated with isolate T1 proved to have positive influence on the total yield of fresh weight.

4. Conclusions

1. The type of fertilisation is a factor that significantly modifies the mycological state of soil.

2. Manure applied to soil caused the greatest proliferation of moulds.

3. The greatest increase in the count of *Trichoderma* sp. was observed in the soil enriched with tomato compost inoculated with strain T2.

4. The application of *Trichoderma* strain T3 in tomato waste compost proved to be the most effective protection of radish from *Fusarium* fungal pathogens.

5. The *Trichoderma* sp. isolates applied in the experiment exhibited a slight antagonism towards *Alternaria* sp. fungal pathogens.

6. The highest yield of radish was noted after the application of tomato compost inoculated with strain T1. It was comparable with the yield obtained after the application of manure.

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