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Distribution of Soil Microorganisms in Field under Potatoes due to Fertilizer and Organics

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ABSTRACT

Background: Using organic manure derived from animal or vegetable matter is often associated with balanced crop production. The influence of organics and fertilizers on soil sustainability during cultivation of potatoes in field was studied.

Methods: Vegetable experiment comprising 10 years (2011-2021), having a control variant (no fertilization), independent mineral, organics (compost) and their combination (50:50%). Changes in the amount and distribution of microbial groups in experimental variants and associated untreated controls were evaluated. Total bacterial number, spore-forming bacteria, fungi, nitrogen-utilizing bacteria and actinomycetes were assessed.

Result: Applied organics and fertilizers had statistically proven a positive effect on growth parameters of potatoes. Organics and fertilizers supplied with mineral and foliar feeding registered taller plants with increased number of branches, number and mass of leaves and stem and root masses, compared to potatoes with organic and organic-mineral combination. Application of compost led to a significant increase in populations of all physiological groups of microorganisms excluding spore-forming bacteria and bacteria utilizing mineral nitrogen, which is a good indicator of increased soil microbial activity in respective treatments. Mineral fertilization had a positive effect on total number of bacteria and on absorption of mineral nitrogen. The treatment in variant (50:50 %) at rhizosphere soil was associated with organic manure application and at non-rhizosphere soil with mineral fertilizer.

Key words: Compost, Mineral fertilizer, Soil microbiota, Soil quality.

INTRODUCTION

The increased requirements for safety of agricultural products and quality of environment determine the dynamic and complex development of plant nutrition technologies. Different additives have been applied for increasing soil fertility for yield increase (Yogananda *et al*. 2019). Factors influence the yield of potatoes or other important food crops (Singh *et al*. 2021) are numerous, but land suitability assessment can lead to decisions on land uses suitable for maximizing crop yield while making best use of it (Singha and Swain, 2020). An alternative to chemicalization in agriculture, an environmentally friendly production is associated with application of organic manure and preparations that provide a more natural environment for plants (Cholakov and Boteva, 2012; Shankaraswamy *et al*. 2017). Organic potato production in many developed countries is a fact (El-Sayed *et al*. 2018, Zorb *et al*. 2014). Due to insufficient information related to application of environmental friendly technologies in this industry, organically produced potatoes are not yet available with in domestic market (Cholakov and Boteva, 2012).

Organic agriculture contributes to mitigating greenhouse gas effect and global warming through its ability to sequester carbon in soil. Different management practices applied in organic agriculture increase the return of carbon to soil, raising crop productivity and favouring carbon storage (Assefa and Tadesse, 2019).

The application of organic manure is undoubtedly associated with a change in the activity of soil microbiota. The presence or absence of substrates activates different processes, which is reflected in the amount and activity of different groups of microorganisms in soil. The possibility of early assessment of efficiency of decomposition of organic components makes it possible to predict and manage the activity of soil microbiota. Soil is a highly heterogeneous medium, comprising mixtures of solid material, of water-filled and of air-filled pores, all of which results in a wide range of proximal microhabitats (Young and Crawford, 2004). Therefore, soil bacteria assign available resources for utilization, as a defence against fluctuating conditions in the surrounding environment (Or *et al*. 2007).

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The aim of the study is to determine the influence of applied organic (compost), mineral and organo-mineral fertilization in combination with foliar feeding with organic and mineral fertilizers on the distribution of soil microorganisms in cultivated soil.

MATERIALS AND METHODS

Field experiments

The experiment was set - up with potato (Soraya) at the end of April 2017, as part of a vegetable crop rotation in experimental station of “N. Poushkarov” Institute, Sofia, Bulgaria.

Scheme of the experimental work

1- Control - without fertilization; 2 - Organic compost (17873 kg/ha); 3 - Mineral fertilization - $N_{20}P_{10}K$; 4 - Mixed fertilization - 50% compost + 50% mineral fertilizer ($N_{10}P_5K_9$).

Fertilization rate of $N_{20}P_{10}K_{18}$ was determined based on the results of a previous study of the team (Mitova and Ruseva, 2014) and literature data (Boteva, 2013; Neshev and Manolov, 2016; Toader *et al*. 2010).

After recalculation based on total nitrogen content of compost, the norm of 200 kg N/ha corresponds to 17873 kg/ha compost was determined. Thus, the fertilized variants were equal in terms of nitrogen content, but not for phosphorus and potassium. In the variant with mineral fertilization, nitrogen was applied in the form of ammonium nitrate, twice ie., first half at the beginning and other half at flower initiation. Phosphorus and potassium were applied before the start of experiment in the form of superphosphate and potassium chloride. The compost used, provided by a licensed producer, was analyzed before the experiment for its composition (Table 1).

During vegetation period, two foliar feeds were made, at flowering and tuber formation phases with foliar fertilizers provided by Lebosol Bulgaria OOD - Lebozol - Potassium and Aminozole. In the second variant, plants were treated twice with Aminozole, in variant 3 with Lebozol-Potassium and in the variant with mixed fertilization - first foliar feeding was with Aminozole and second with Lebozol-Potassium. Lebozol-Potassium is a fertilizer solution with pH value of 14, containing 31% water-soluble potassium (465 g/l K_2O) and 3% amide nitrogen (45 g/l N). It was used as 1% solution at a rate of 500 ml/da. It improves the quality of produce and has plant protection effect. Aminozole is registered under Bulgarian law as Organic Fertilizer. It is a water-soluble, environmentally friendly, yellow-brown thick liquid with pH value between 5 and 7. It contains more than 20 different amino acids and peptides (56-58%) and 9.4% N (116 g/l) organically bound nitrogen. In potatoes it improves the absorption of nutrients, root and tuber formation, strengthens immunity and it is applied at the 0.2-0.3 l/da.

Table 1: Important parameters of compost used in the present study.

	Units	Values
Total nitrogen	mg/g	28, 8±2, 9
Dry matter	%	30, 73±3, 07
$N\text{-}NO_3$	mg/kg	3, 65±0, 36
$N\text{-}NO_4$	mg/kg	39, 8±4, 0
Nitrogen (Kjeldahl method)	g/kg	28, 8±2, 9
TOC	g/kg	496±50
Soluble forms		
Phosphorus (P_2O_5)	mg/kg	12911
Potassium (K_2O)	mg/kg	2083
Sulphur from sulphates	mg/kg	271

The content of macro and microelements in soil was determined by standard methods (Arinushkina, 1970). Total nitrogen by Kjeldahl method (FAO, 2017), ammonium and nitrate nitrogen, mobile forms of phosphorus and potassium by colorimetrically; pH is by potentiometrically, in aqueous extract and potassium chloride solution.

Microbiological characteristics

The development of soil microorganisms was assessed in all treatments of the experiment during late flowering. Samples were taken from a depth of 0-20 cm after clearing the residues. The total bacterial number, spore-forming bacteria, fungi, nitrogen-utilizing bacteria and actinomycetes were assessed (Alef and Nannipieri, 1998; Goushterov *et al*. 1977). After the analysis, the moisture content of each soil sample was determined and the results were recalculated and presented as the amount of dry soil. All microbiological analyses were made in the Agrarian University- Plovdiv, Bulgaria.

RESULTS AND DISCUSSION

In crop rotation, potatoes are planted after the predecessor crop, beans. The experimental station is located in Plovdiv district (South Central Bulgaria). The soil is Alluvial-meadow-having slight humus with mildly alkaline reaction (pH in water 7.2-7.3). The residual content of mineral nitrogen, despite the fact that the predecessor crop was nitrogen - fixing legume is low, between 21.5 and 24.8 mg/kg soil (Table 2). The content of mobile phosphorus varies from low (11.3 mg/100g soil) in unfertilized variant to considerably high (42.8 mg/100g soil) in variant with organic fertilization. The mobile potassium in soil of experimental variants varies slightly and signifies the experimental area as a medium stock (14.6-18.9 mg/100g soil) under this nutrient.

The cluster analysis by indicators (columns) singled out 2 clusters, the first includes humus, P_2O_5, K_2O and Σ $N\text{-}NH_4 + NO_3$ and the second pH (Fig 1). The cluster analysis by variants (rows) separated 2 clusters, in the first variant 2 and 4 fall and in the second variant 1, 3. According to indicators (columns), there are 2 clusters (Fig 2). The first includes K_2O and Σ $N\text{-}NH_4 + NO_3$ and the second is humus, P_2O_5 and pH. By variants (rows), 2 clusters were separated in the first variant 2 and 4 fall and in the second variants 1 and 3.

Table 2: Soil characteristics, before the experiment.

Treatments	pH(H_2O)	pH (KCl)	ΣN-NH_4+NO_3	P_2O_5	K_2O	Humus
				mg.100 g^{-1}		%
Control	7.3	6.8	21.5	11.3	18.1	1.71
100% compost	7.3	6.9	21.3	42.8	14.8	1.71
100% mineral fertilizer	7.2	6.7	10.9	18.0	18.9	1.53
50% compost + 50% mineral fertilizer	7.3	6.9	24.8	26.8	14.6	1.54

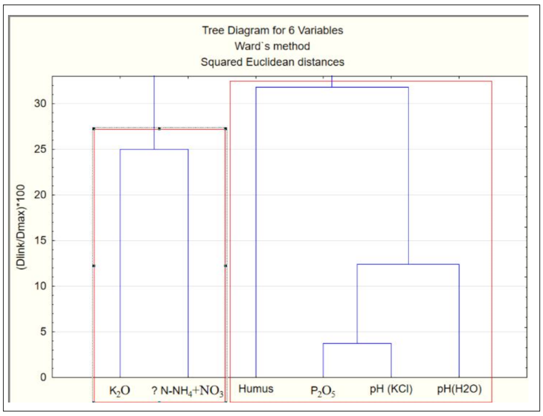

Fig 1

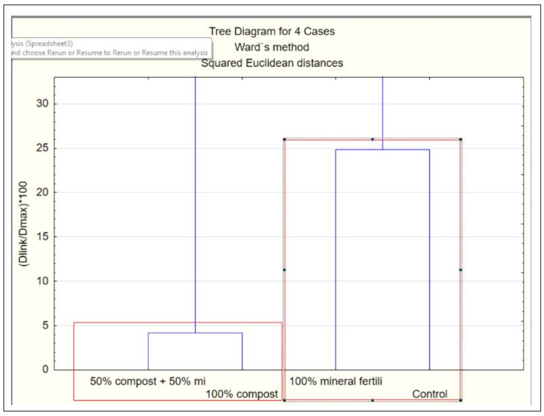

Fig 2

Fig 1 and 2: Correlation among soil parameters and variants.

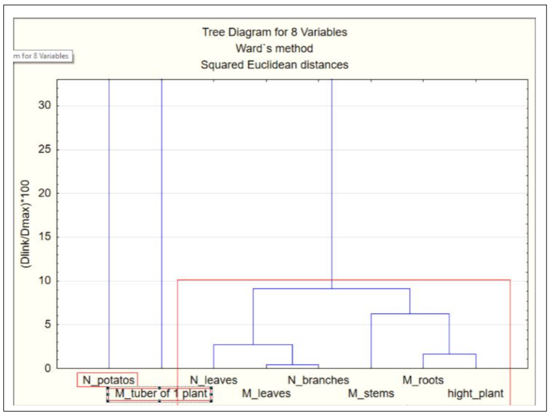

Fig 3

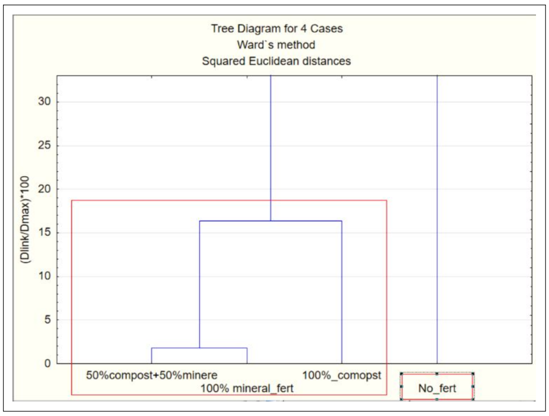

Fig 4

Fig 3 and 4: Influence of fertilization on biometric indicators of potatoes in the phase of tuber formation.

Biometric data

At the time of reporting in the beginning of tuber formation phase, the average number of tubers per plant is between 4. 3 and 6.0. Although there are proven differences between the number of tubers between plants with mixed fertilization (6.0 pieces) and other variants, it should be noted that at-least (4.3 pieces) that formed tubers are plants supplied with organic fertilization and nutrition, which can be explained by the slow pace of plant development as a result of the slower mineralization of organic matter, compared to the variants with mineral and organo-mineral fertilization. There are statistically proven differences in the mass of tubers of one plant between plants with mineral fertilization and those without fertilization, as well as with those with organic and mixed fertilization (Fig 3).

The cluster analysis by indicators (columns) singled out 3 clusters, the first of which includes only the number of potatoes, the second is the mass of tubers from one plant, while the third includes biometric indicators - number of leaves, leaf mass, stem mass, mass of roots and height of plants. The cluster analysis by variants (rows) singled out 2 clusters, the first of which included variants 2, 3 and 4 (fertilizers) and the second - only variant 1 (control).

Both organic manure and inorganic fertilizers had their role in soil properties and agriculture and good influence of each should be pointed and used appropriately, according to the effect sought (Kakar *et al.* 2019).

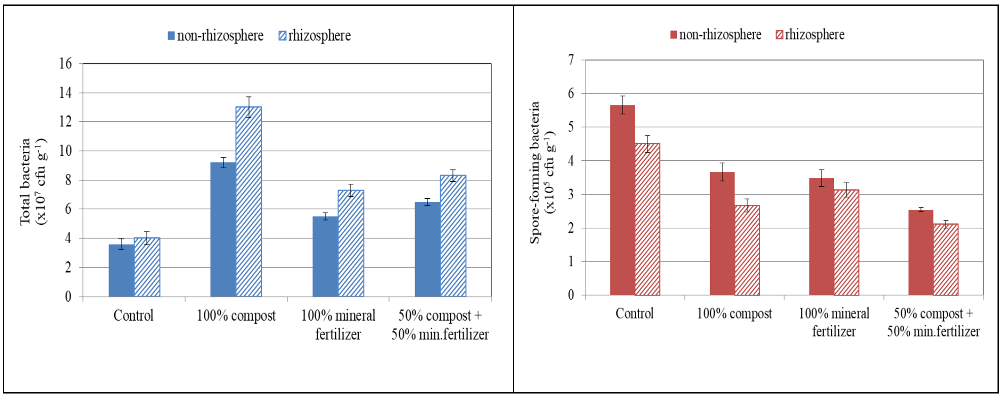

Fig 5: Number of colony forming units (*cfu*) of total bacteria (left) and spore-forming bacteria (right) in rhizosphere and non-rhizosphere soils due to the treatments. The data express the mean and standard error of three repetitions.

Microbial characteristics

At this stage, predetermining the activity of tuber formation, a large set of microbiological parameters was measured, allowing to make an early assessment of efficiency of fertilization. The development of soil microbial communities is regulated by various ecological laws. Their requirements for the environment, the competitive relationships between different soil populations are reflected in the adaptive changes that determine their distribution and their ability to survive. The application of organic and /or mineral fertilizers in soil is a factor that has a direct impact on entire soil microbiome. The same fact was reported by Choudhury *et al.* (2019).

The total number of viable bacteria showed some changes between treatments of the experiment (Fig 4). However, their quantity is relatively lowest in the control, without incorporation of additives. The most significant increase compared to the control was found in the treatment with application of compost. This was due to the presence of organic substances that stimulated the development of heterotrophic bacterial communities in soil. Significant differences in the number of bacteria between the treatments with application of 100% mineral or combined organic-mineral fertilization was not found. Nunan *et al*. (2020) discussed this phenomenon, how the heterogeneity of soil microbial environment and the consequent uncertainty associated with acquiring resources, may influence how microbial metabolism, motility and interactions evolved. Ultimately, the overall microbial activity that is represented in ecosystem models, such as heterotrophic decomposition or respiration. Their analysis of utilization pathways showed that some phyla (Proteobacteria and Actinobacteria) tended to have more pathways than the other phyla, suggesting a broader resource use capacity.

As a rule, spore-forming bacteria form spores in the presence of adverse conditions *viz.*, lack of nutrients or moisture, presence of harmful effects, *etc.* The decreases in their numbers indicate a partial improvement of conditions for development in soil habitats. This is the case of the treatments applied with compost, whose organic molecules are used as a source of carbon and energy from heterotrophic or as a source of carbon from hemolitotrophic microbial populations. The highest number of spore-forming bacteria was found in control, while the lowest was in treatment with combined fertilization. A small decrease of amount of these bacterial groups in the rhizosphere of all treatments compared to the non-rhizosphere was found with exception of the treatment with mineral fertilization.

The application of mineral fertilizers has a different effect on the different physiological groups of microorganisms. In our case, the application of 100% of mineral fertilizer led to an increase in the populations of bacteria utilising mineral nitrogen in non-rhizosphere soil, while those in the rhizosphere were not affected (Fig 5, left). The number of these populations in compost treatments marked a slight decrease.

Different microbial communities dominate during composting phases, starting with bacteria, the most important decomposers due to their rapid growth rates. Not less is the role of actinomycetes, which prefer moist conditions with neutral or slightly alkaline pH. Actinomycetes are microorganisms synthesizing a large number of enzymes, including exoenzymes. They use short-chain organic substances as a carbon source, but also polymers,

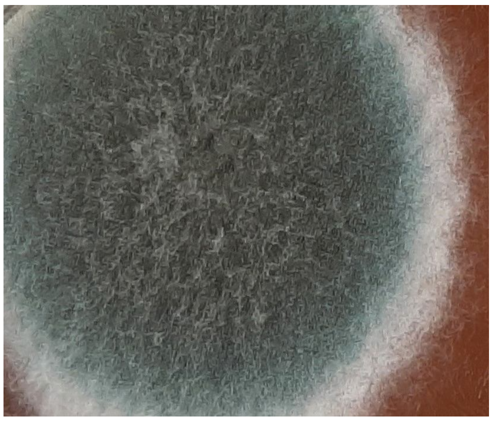

Fig 6: Digital image of fungi from soil with 100% compost in a petri-dish with: potato-glucose agar.

while they use various organic and inorganic compounds as a nitrogen source. The application of compost and mineral fertilization led to an increase in the amount of actinomycetes compared to control. The most significant increase was registered under the non-rhizosphere soil of the treatment with combined fertilization (50% compost + 50% mineral fertilizer) and in the rhizosphere of the treatment with 100% compost (Fig 5, right). The *cfu* of actinomycetes were less in the treatment with 100% mineral fertilization, with a more significant increase observed in the rhizosphere. In general, organic fertilization had a stronger stimulating effect on the development of actinomycete's populations than mineral fertilization. They play major roles in cycling of organic matter, inhibit the growth of some plant pathogens in the rhizosphere and decompose complex mixtures of polymers of plant, animal and fungal material by the production of many extracellular enzymes, which are conductive to crop production (Bhatti *et al*. 2017). Although, actinomycetes were in smaller numbers compared to ammonifying bacteria and bacteria utilising mineral nitrogen, their amount is significant compared to that found in other (Ivanova *et al*. 2019).

Fungi are eukaryotes that synthesize various extracellular enzymes. Fungi are well adapted for decomposition processes due to their filamentous growth and extracellular nutrition (Wright *et al*. 2016). Fungi and actinomycetes are thought to underlie the formation of soil humus. The fungi, as heterotrophs, are able to degrade various biopolymers, that is why they are of particular interest in the present study (Fig 6). Application of compost led to a significant increase in the number of fungi compared to control. In treatment with 100% compost, they were more than three times higher compared to control and to mineral fertilization treatments, while the increase in treatment with mixed fertilization was twice (Fig 7, right). These differences are explained by the degradation of organic matter from compost by the fungi.

The number of cellulose degrading soil bacteria was influenced by the organic fertilization (Fig 8, right). In the non-rhizosphere of compost treatment, their amount was over 4 times higher compared to control and in the rhizosphere soil, the amount reached 8.10^3cfu/g. This was 100% higher than non-rhizosphere of the same treatment and 7 folds higher than control. A smaller increase was observed in case of combined fertilization, 3.5 folds compared to non-rhizosphere in control and more than 4 folds compared to the amount of these bacteria in the rhizosphere.

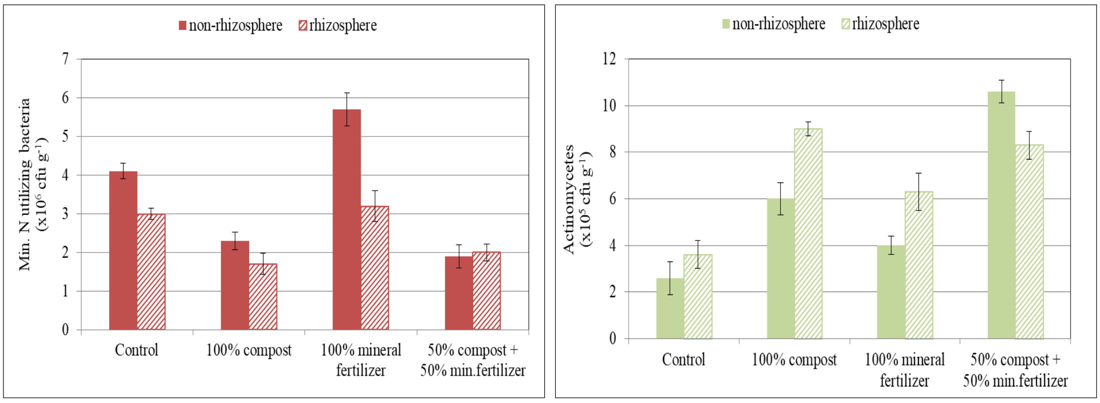

Fig 7: Number of colonies forming units (cfu) of bacteria utilizing mineral nitrogen (left) and actinomycetes (right) in the rhizosphere and non-rhizosphere in different variants of the experiment. The data express the mean and standard error of three repetitions.

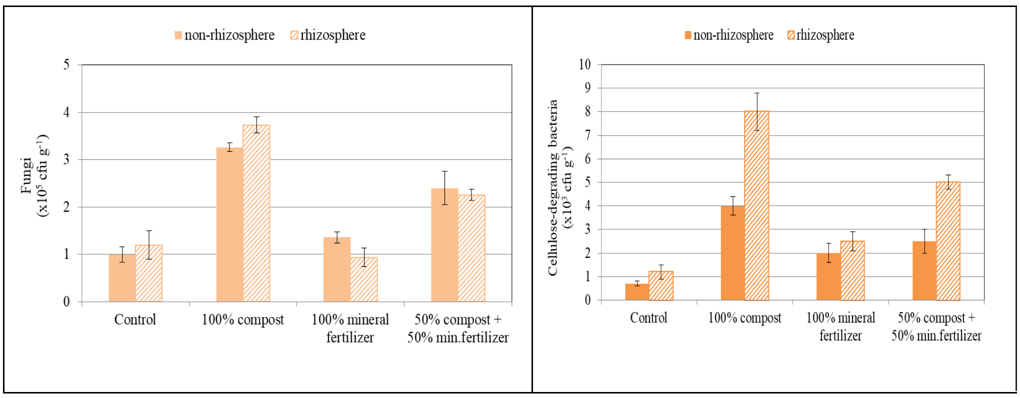

Fig 8: Number of colonies forming units (*cfu*) of fungi (left) and cellulose-degrading bacteria (right) in the rhizosphere and non-rhizosphere in different variants of the experiment. The data express the mean and standard error of three repetitions.

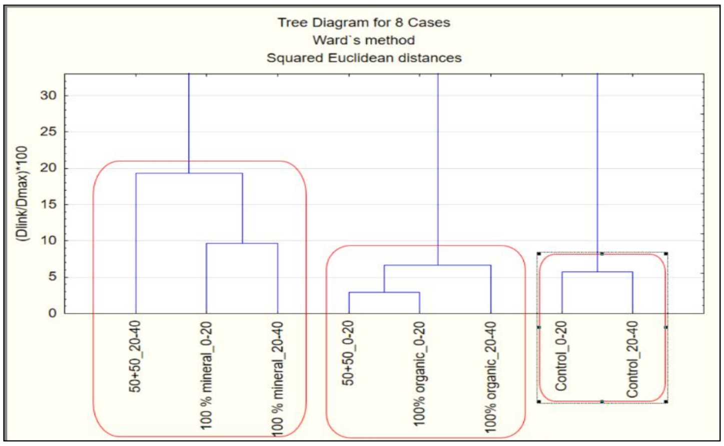

Fig 9: Cluster analysis of treatments.

Under the cluster analysis, treatments are forming three clusters (Fig 9). The first includes the sites of 100% mineral fertilizer and the variant 50 + 50 at soil samples from non-rhizoshere site, the second cluster includes rhizoshere site of 100% organics and the variant 50 + 50 at from non-rhizoshere site, the third cluster covers the two sites of control.

CONCLUSION

The applied fertilization has a statistically proven positive effect on growth indicators of potatoes. Plants with mineral fertilization and foliar feeding form taller plants, with a larger number of branches, number and mass of leaves, stem and root masses, compared to potatoes under organic and organic-mineral fertilization.

The application of compost has led to a significant increase in the populations of all physiological groups of microorganisms with exception of spore-forming bacteria and bacteria that utilize mineral nitrogen, which is a very good indicator of increased soil microbial activity in the respective treatments. In addition, mineral fertilization had a positive effect on total number of bacteria and in absorption of mineral nitrogen.

We can conclude that the treatment 50% organic+ 50% mineral fertilizers at rhisosphere soil is associated with organic fertilizer and at non-rhizophere soil with the mineral fertilizer.

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