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Colonization of 'Sampion' apple tree roots and rhizosphere by mycorrhizal fungi following the application of AquaGel or organic compost enriched with beneficial microorganisms

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Abstract: Apple trees of the cultivar 'Sampion' were planted at the Experimental Orchard of National Institute of Horticultural Research in Dąbrowice. In a three-year experiment (2019−2021) the following fertilization combinations were applied: AquaGel (commercial hydrogel), AquaGel combined with a consortium of beneficial bacteria, AquaGel combined with a consortium of beneficial filamentous fungi, organic compost alone, compost combined with a consortium of beneficial bacteria, and compost combined with a consortium of beneficial filamentous fungi. The presence of arbuscular mycorrhizal fungi was assessed both in the rhizosphere soil and in the roots of apple trees. The results of the study indicate the most advantageous effect of compost combined with bacteria or fungi on the degree of root colonization by arbuscular mycorrhizal fungi and the formation of their spores in the soil.

Keywords: fertilization; mycorrhizal frequency; soil; spores; beneficial bacteria; beneficial fungi

The depletion of organic matter in arable soils in Poland, observed in recent decades, is a serious problem in the cultivation of horticultural and agricultural plants. Not only does low soil organic matter content cause a decrease in soil fertility and an inadequate supply of plants with minerals, but also an increased in susceptibility of soils to erosion and degradation. On the other hand, there are growing demands of consumers who require food that is not only healthy and safe, free from residues of agrochemicals, but also produced in a manner that is safe for the environment, human health and animal welfare. For this reason, there is an urgent need to reduce the use of mineral fertilizers and other chemical plant production inputs, more effective use of safe means of production, and the implementation of biofertilization in plant crops. Another important factor influencing agricultural production is the withdrawal of further active substances, which limits the availability of effective and safe plant production means to farmers (Hillocks 2012; Sawinska et al. 2020). Environmental protection requirements limit the use of mineral fertilizers, in particular nitrogenous ones. All these elements may result in a decline in agricultural production, and also in further degradation of agricultural soils. For this reason, rapid interven-

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tion measures are required. One such solution is to improve the quality of soils by enriching them with soil humus by providing organic substances in the form of peat, manure, compost, humic acids or green fertilizers. Some of these materials enrich the soil not only with organic matter, but also with numerous minerals. These measures improve the soil structure and increase its resistance to degradation (De Corato 2020; Ravindran et al. 2021). However, in the case of severely degraded soils with low microbiological activity, such measures are not able to restore the proper functioning of the soil microbiome.

Therefore, bio-fertilizers and microbiologically enriched composts have a decisive advantage over the above-mentioned methods. They are innovative products enriched with beneficial microorganisms, acting as biostimulants, fertilizers and protective measures against soil and plant pathogens. Moreover, they improve soil fertility by supplying organic matter (compost) that undergoes gradual mineralization and humification in the soil, which thus becomes a source of nutrients for plants. Their addition improves the soil structure and increases the diversity of soil microorganisms (Bonanomi et al. 2020). According to Kobus (1995), they are in the soil multilevel interactions between the components of the microbiome, organic matter and plants. The breakdown of organic matter leads to changes in soil structure and biology. Biologically active compounds influence plant cell division, soil and plant microbiome, as well as bio-physico-chemical processes taking place in the rhizosphere, e.g. mycorrhizal associations with plant roots (Aghhavani et al. 2018). Decomposition of soil organic matter occurs mainly with the participation of soil microorganisms. Plant roots are colonized mainly by Gram-negative bacteria, e.g. of the genus *Pseudomonas* (Beneduzi et al. 2012), which increase plant resistance to pathogenic nematodes and stimulate nutrient uptake. Gram-positive bacteria of the genus *Bacillus* are also the most common soil-inhabiting bacteria. Their role is to break down organic compounds of plant origin, mainly carbohydrates and pectins (Radhakrishan et al. 2017; Hashem et al. 2019). Another group of microorganisms, important from the point of view of maintaining the proper condition and structure of the soil and plant productivity, are mycorrhizal fungi.

Arbuscular mycorrhizal fungi play an important role in the mineral nutrition of plants, their growth, yielding and protection against biotic and abiotic soil factors. Their importance is particularly evident during periods of drought and in soils with a low nutrient content. This is because the hyphae of their mycelium form a several-meter-long network in the soil, helping plants to obtain nutrients and water from the soil (Mannino et al. 2020). Arbuscular mycorrhizal fungi, through symbiotic interactions with plants, play an important role in protecting plants against abiotic stresses such as: deficiency/excess of nutrients, high/low temperatures, and drought (Chitarra et al. 2016; Wu 2017; Volpe et al. 2018). Under stressful conditions, the presence of the mycelia of arbuscular mycorrhizal fungi (AMF) enables more intensive uptake of water and mineral ions by crop plants (Moucheshi et al. 2012). Arbuscular mycorrhizal fungi have been found to increase the induced tolerance to water shortage through symbiotic interactions with crop plants, including improved conductivity of the stomata (Augé et al. 2015), increased water-use efficiency, and reduced oxidative damage (Pedranzani et al. 2016). This happens because arbuscular mycorrhizal fungi take up and make available to plants through mycelial hyphae the available forms of N, P, K and micronutrients and water, and use some mechanisms to prevent and reduce diseases caused by soil pathogens (Widnyana et al. 2019). Arbuscular mycorrhizal fungi, due to their beneficial effects on plants, are used as components of bio-fertilizers and other biopreparations used in plant cultivation (Mosa et al 2014; Abdel-Wahab 2018).

Biofertilizers are environmentally friendly and economically viable. They are an effective alternative to standard NPK fertilization. They are recommended for growing plants and are also and are effectively used for bioremediation of exhausted cultivated soils. The use of bio-fertilizers increases the soil organic matter content by at least 50% and causes the release of assimilable forms of mineral ions, increasing their concentration in the soil by up to 100% (Riaz et al. 2020). A significant advantage of using bio-fertilizers is the increase in the microbial activity of the soil, including the increase in the population of beneficial soil microorganisms. Innovative bio-fertilizers show synergistic multifunctional effects: hormonal, biostimulating, fertilizing, and protective (Aggani 2013; Kumar et al. 2018).

The aim of the study was to assess the degree of colonization of 'Sampion' apple tree roots and rhizosphere by arbuscular mycorrhizal fungi following the application of organic compost enriched with beneficial microorganisms.

https://doi.org/10.17221/1/2023-HORTSCI

MATERIAL AND METHODS

Experimental conditions. The trees of the cultivar 'Sampion' grafted on M. 9 rootstock were planted in the spring of 2019 in the Experimental Orchard of National Institute of Horticultural Research (IO) in Dąbrowice on a podzolic soil of valuation class 3b at a distance of 4 m × 2 m, in a random block design, in 4 replications (one experimental plot = 8 m^2). For the three years of the study (2019–2021), the trees were maintained according to the standard apple-tree cultivation system. During periods of drought, or long times without rain (Figure 1), the trees were drip irrigated. Protection against pests and diseases was performed in accordance with the Fruit-Plant Protection Programme.

The following treatments were applied:
1. Negative control – no fertilization.
2. Positive control – NPK fertilization, applied before and after planting: 20 g of Super Fos Dar 40, 100 g of potassium salt, 20 g of urea/tree.
3. AquaGel alone, applied only during planting at 100 g/tree.
4. AquaGel applied together with a consortium of beneficial bacteria at 3.83 g/tree (the consortium consisted of strains: CZP4/4 *Priestia* sp., AF75BB *Bacillus amyloliquefaciens*, CHT114AB *Paenibacillus polymyxa*).
5. AquaGel applied together with a consortium of beneficial filamentous fungi at 5.25 g/tree (the consortium consisted of strains: G119AA *Aspergillus niger* and WT15A *Purpureocillium lilacinum*).
6. Organic compost (alone).
7. Organic compost applied together with a consortium of beneficial bacteria at 3.83 g/tree (the consortium consisted of strains: CZP4/4 *Priestia* sp., AF75BB *Bacillus amyloliquefaciens*, CHT114AB *Paenibacillus polymyxa*).
8. Organic compost applied together with a consortium of beneficial filamentous fungi at 5.25 g/tree (the consortium consisted of strains: G119AA *Aspergillus niger* and WT15A *Purpureocillium lilacinum*).

Preparation of fertilizers. 100 g of AquaGel powder was diluted in 2 L of water, then the tree roots were immersed for one minute and planted. Organic compost in the amount of 5 liters

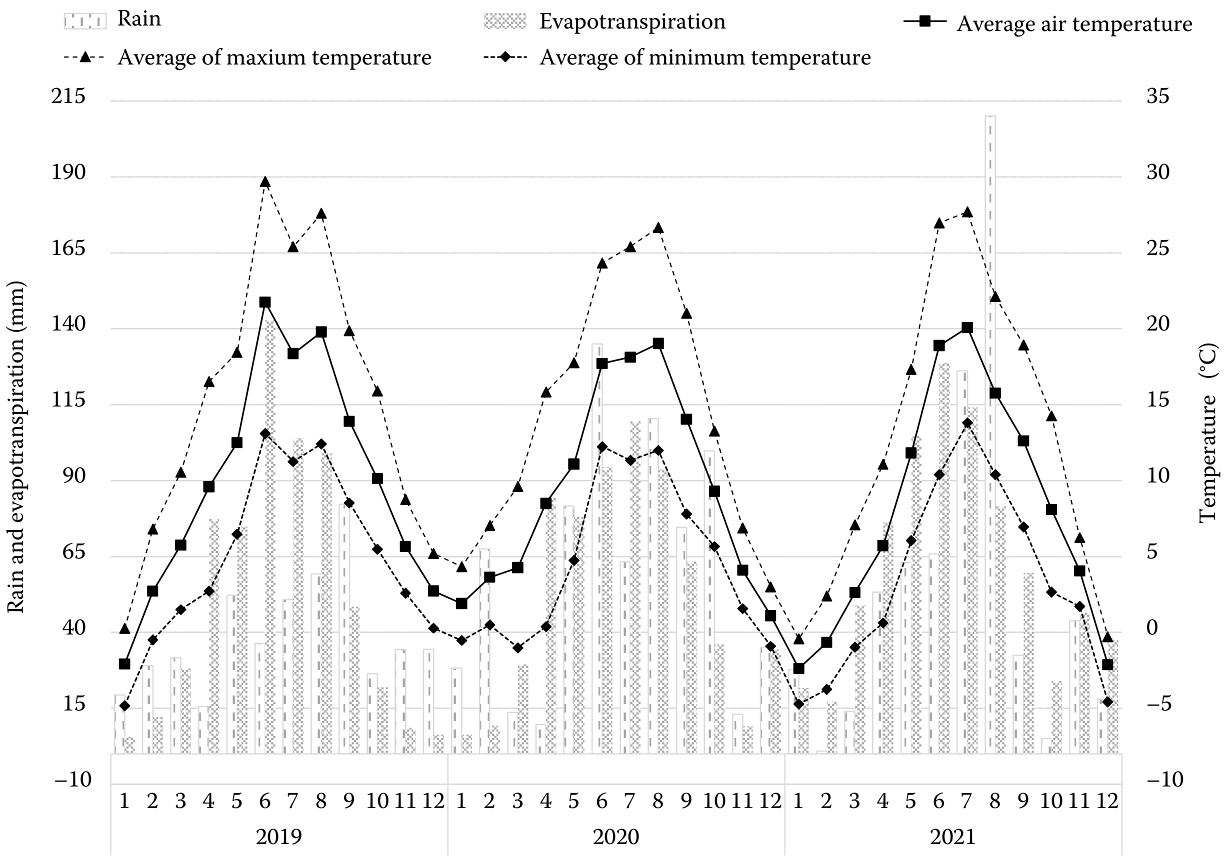

Figure 1. Thermal and humidity conditions at the experimental site (data from a weather station located in the Experimental Orchard)

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was applied during planting and in the next two years of the experiment. During planting, organic compost was applied in a 5-litre dose directly under the tree, having been mixed beforehand with the soil. In the following years of the experiment (2020, 2021), compost was applied around the trees in 5-litre doses.

The microorganisms combined with AquaGel or with the compost were applied during planting in 2019 and in the next two years of the experiment. In the spring, microbiological inocula in loose form (bacteria at 3.83 g/tree, fungi at 5.25 g/tree) were mixed with the topsoil immediately after planting the trees in the experimental plots and in the spring in the following years of the experiment. In the NPK control combination, mineral fertilizer Super FOS DAR 40 was applied around the trees and mixed with the topsoil during planting and in the following years of the experiment. All fertilization treatments were repeated in the second and third years of the experiment.

Characteristics of the fertilizers used. Super FOS DAR 40 contains 40% P_2O_5 – phosphorus pentoxide soluble in mineral acids; 25% P_2O_5 soluble in neutral citrate solution and water; 10% CaO – calcium oxide soluble in water, and microelements (Co, Cu, Fe, Mn, Zn), derived from natural phosphates (produced by Grupa Azoty S.A.).

AquaGel contains 0.64% N, 0.43% K_2O (produced by Kozielski Sp. z o.o. in the form of a powder, strongly absorbing water).

Organic compost contains 8.7% humus, 0.51% nitrogen NO_3, 0.30% phosphorus, 0.45% potassium, 15% magnesium, (produced by Zakład Przetwórstwa Owocowego Doehler Sp. z o.o).

Characteristics of the microorganisms used. The bacterial strains CHT114AB (*Paenibacillus polymyxa*), CZP4/4 (*Priestia* sp.) and the fungus strain G119AA (*Aspergillus niger*) were selected from the rhizosphere soil of wheat from Ukraine. The strain AF75BB (*Bacillus amyloliquefaciens*) was selected from the rhizosphere soil of the grapevine cv. Regent on the 5BB rootstock in the Experimental Orchard of the National Institute of Horticultural Research. The *Purpureocillium lilacinum* (SYMBIOBANK strain number: WT15A) was isolated from the biomass collected from the water bath.

Preparation of inocula. The bacterial inocula were prepared by Skotan S.A. company, in a spray drying process with the use of maltodextrin (with dextrose equivalent 7−13) as a bulking agent. The concentration of bacteria was: AF75BB *Bacillus amyloliquefaciens* 2, 3 × 10^7 cfu/g, CZP4/4 *Priestia* sp. 2, 4 × 10^9 cfu/g, CHT114AB *Paenibacillus polymyxa* 9, 5 × 10^9 cfu/g.

The fungi inocula were prepared in a solid fermentation process. The fungi were cultivated on rice (G119AA *Aspergillus niger*) or on cornmeal (WT15A *Purpureocillium lilacinum*) for two weeks. The overgrown substrates were dried in the oven at 39 °C and then they were pulverized and mixed in the ratio 1 : 8 (G119AA *Aspergillus niger* : WT15A *Purpureocillium lilacinum*). The final concentration was approx: 1–2 × 10^8 cfu/g.

Determination of rhizosphere colonization by arbuscular mycorrhizal fungi . The soil from the rhizosphere of the apple trees was collected in July of each year; 100 g samples were taken for analysis. Each sample was placed in 1 litre plastic bottles and suspended with tap water (0.5–1 L of water). The material was thoroughly mixed for 5 minutes and placed in a refrigerator (at 4 °C). After 24 hours, the mixture was poured through a column of sieves with pores of the following diameters: 1.0, 0.5, 0.125, 0.063 and 0.045 mm. Next, 5 ml of collected material from each sieve was transferred to petri dishes (120 mm) by pouring tap water over the sieve. Sucrose was dissolved in each dish (5 g per dish) so that the spores would float to the surface (Błaszkowski 2003; 2008). The prepared samples were examined under a Nikon SMZ 800 stereoscopic microscope. AMF spores (chlamydospores) present in the water were counted.

Determination of root colonization by arbuscular mycorrhizal fungi. The fine roots of apple trees (10 g from each replication), collected in July of each year, were stained according to the method developed by Derkowska et al. (2015a). Microscopic specimens were prepared and examined with a Nikon Eclipse 50i microscope (objectives with magnifications of 20×, 40×, 60×, 100×), and photographic records of the observed mycorrhizal structures were made. The assessment of the degree of colonization of the roots by arbuscular mycorrhizal fungi was performed by the method of Trouvelot et al. (1986). Based on the results, mycorrhizal frequency (*F*%), relative and absolute mycorrhizal intensity (*M*, *m*%), and relative and absolute abundance of arbuscules (*A*, *a*%) were calculated using the computer program MYCOCALC, available from the website: http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/MYCOCALC.EXE

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Table 1. Influence of composts and beneficial microorganisms on the degree of root colonization of 'Sampion' apple trees by arbuscular mycorrhizal fungi. Results for the year 2019

Treatment	*F* (%)	*M* (%)	*m* (%)	*a* (%)	*A* (%)
Control (no fertilization)	8.89[a]	0.87[b]	9.00[b]	0	0
NPK	8.89[a]	0.22[a]	2.0[a]	0	0
AquaGel	10.0[ab]	0.88[b]	8.78[b]	0	0
AquaGel + Bacteria	14.44[c]	1.29[c]	8.97[b]	0	0
AquaGel + Fungi	12.22[bc]	1.24[c]	10.16[b]	0	0
Organic compost	17.78[d]	1.73[d]	9.60[b]	0	0
Organic compost + Bacteria	18.89[d]	1.74[d]	9.19[b]	0	0
Organic compost + Fungi	17.78[d]	1.37[c]	7.72[b]	0	0

F – the mycorrhizal frequency; *M* – relative mycorrhizal intensity; *m* – absolute mycorrhizal intensity; *a* – absolute abundance of arbuscules; *A* – abundance of arbuscules

[a–d]Means in columns marked with the same letter do not differ significantly at $P = 0.05$ according to Tukey's multiple test

In addition, the course of the weather in 2019–2021 was monitored, i.e. precipitation, evapotranspiration, and the average, maximum and minimum temperatures.

Statistical analysis. The results were statistically analyzed by one-way analysis of variance in a random block design. Multiple comparisons of means for the combinations were performed with Tukey's test at a significance level of $\alpha = 0.05$ using STATISTICA v.10 software (StatSoft Inc. 2011).

RESULTS

The highest average air temperature (22 °C) in the Experimental Orchard of the National Institute of Horticultural Research was recorded in June 2019. The average and maximum air temperatures were also high in June and July of 2021, and were 2–3 °C higher than in 2020 (Figure 1). Moreover, in August 2021, a high level of rainfall (30 mm) was observed, which could have had an additional impact on the measurements (Tables 1–3).

The results of the study indicate beneficial effects of the applied compost, AquaGel and microorganisms used on the degree of root colonization by arbuscular mycorrhizal fungi and the formation of AMF spores in rhizosphere soil, in the field cultivation of 'Sampion' apple trees.

The effects on the degree of root colonization and the formation of AM fungi depended on the type of fertilization (AquaGel or compost), the consortium of beneficial microorganisms (bacteria or fungi), and the year of the study.

Both in the first and the second year of the experiment, the use of compost alone or compost together with the bacterial or fungal consortium increased the colonization of apple tree roots by arbuscular

Table 2. Influence of composts and beneficial microorganisms on the degree of root colonization of 'Sampion' apple trees by arbuscular mycorrhizal fungi. Results for the year 2020

Treatment	*F* (%)	*M* (%)	*m* (%)	*a* (%)	*A* (%)
Control (no fertilization)	10.0[ab]	0.88[a]	8.78[a–c]	0	0
NPK	8.89[a]	0.87[a]	9.00[a–c]	0	0
AquaGel	14.44[cd]	1.33[bc]	9.30[a–c]	0	0
AquaGel + Bacteria	16.67[d]	1.35[bc]	8.27[ab]	0	0
AquaGel + Fungi	12.22[bc]	1.22[b]	10.17[bc]	0	0
Organic compost	20.0[e]	1.53[cd]	7.61[a]	0	0
Organic compost + Bacteria	23.33[f]	2.48[e]	10.73[c]	0	0
Organic compost + Fungi	21.11[ef]	1.77[d]	8.17[ab]	0	0

F – the mycorrhizal frequency; *M* – relative mycorrhizal intensity; *m* – absolute mycorrhizal intensity; *a* – absolute abundance of arbuscules; *A* – abundance of arbuscules

[a–f]Means in columns marked with the same letter do not differ significantly at $P = 0.05$ according to Tukey's multiple test

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Table 3. Influence of composts and beneficial microorganisms on the degree of root colonization of 'Sampion' apple trees by arbuscular mycorrhizal fungi. Results for the year 2021

Treatment	*F* (%)	*M* (%)	*m* (%)	*a* (%)	*A* (%)
Control (no fertilization)	1.11[a]	0.01[a]	0.33[b]	0	0
NPK	0[a]	0[a]	0[a]	0	0
AquaGel	28.89[e]	0.88[d]	2.70[f]	0	0
AquaGel + Bacteria	15.55[cd]	0.24[b]	1.56[d]	0	0
AquaGel + Fungi	13.33[bc]	0.13[ab]	1.00[c]	0	0
Organic compost	12.22[b]	0.53[c]	4.19[h]	0	0
Organic compost + Bacteria	17.77[d]	0.35[bc]	1.98[e]	0	0
Organic compost + Fungi	15.55[cd]	0.52[c]	2.94[g]	0	0

F – the mycorrhizal frequency; *M* – relative mycorrhizal intensity; *m* – absolute mycorrhizal intensity; *a* – absolute abundance of arbuscules; *A* – abundance of arbuscules

[a–h]Means in columns marked with the same letter do not differ significantly at $P = 0.05$ according to Tukey's multiple test

mycorrhizal fungi to the greatest extent (Tables 1 and 2). In the third year of the experiment, compost enriched with a bacterial or fungal consortium had a more favourable effect on the presence of AM fungi in apple roots than compost alone and compared to the negative and positive controls. In the third year of the experiment, the application of AquaGel alone during planting had the greatest effect on increasing the colonization of apple roots by mycorrhizal fungi, compared to the roots of positive and negative control plants (Table 3). During the three years of the experiment, the use of bioproducts applied alone or enriched with microorganisms increased the relative intensity of the mycorrhiza compared to the negative and positive controls. The positive effect of the bioproducts applied alone or enriched with microorganisms at the absolute intensity of AM fungi compared to the negative and positive controls was observed in the third year of the experiment.

The stimulating effect of microorganisms on the colonization of plant roots by arbuscular mycorrhizal fungi was visible after the application of AquaGel with bacteria in the first year of the experiment and after the application of organic compost with bacteria or fungi in the second and third years of the experiment compared to AquaGel and compost applied without microorganisms. No significant differences were found between bacterial and fungal inocula for the colonization of plant roots with mycorrhizal fungi, except for the year 2020, where bacterial strains in combination with AquaGel stimulated the presence of AM fungi in plant roots to a greater extent. For two seasons, 2019 and 2020, no significant differences were found in the number of AMF spores in the soil of apple trees under the influence of applied fertilization, compared to the positive and negative control (Table 4). In the third year of the experiment, a significantly greater number of spores

Table 4. Influence of compost and beneficial microorganisms on the presence of spores of arbuscular mycorrhizal fungi in the rhizosphere soil of 'Sampion' apple trees (Experimental Orchard of the National Institute of Horticultural Research in Dąbrowice, 2019–2021)

Treatment	Number of spores in 100 g of soil		
	2019	2020	2021
Control (no fertilization)	0[a]	3[a]	5[ab]
NPK	0[a]	1[a]	2[a]
AquaGel	3[a]	5[a]	7[ab]
AquaGel + Bacteria	5[a]	9[ab]	11[b]
AquaGel + Fungi	2[a]	5[a]	7[ab]
Organic compost	5[a]	8[a]	10[b]
Organic compost + Bacteria	7[ab]	10[ab]	13[b]
Organic compost + Fungi	4[a]	7[a]	10[b]

[a,b]Means in columns marked with the same letter do not differ significantly at $P = 0.05$ according to Tukey's multiple test

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was found in the rhizosphere soil after treatments with AquaGel + bacteria and in combinations with compost (organic compost, organic compost + bacteria, organic compost + fungi) compared to the positive control.

DISCUSSION

In the research conducted, a positive effect of AquaGel, compost and beneficial microorganisms on the degree of apple tree root colonization and on the number of AM fungi spores in the soil was observed.

The application of organic compost and AquaGel enriched with microorganisms had a positive effect on the colonization of apple tree roots by AM fungi during the three-year period of the experiment. Organic compost, after combined application with beneficial bacteria and fungi, doubled the degree of mycorrhizal association in the roots of apple trees compared to control plants (2019: Control NPK – 8.89 F%, Organic compost + Bacteria – 18.89 F%; 2020: Control NPK – 8.89 F%, Organic compost + Bacteria – 23.33 F%).

The bioproducts used alone or enriched with microorganisms also increased the relative intensity of the mycorrhiza compared to the negative and positive controls over the three years of the experiment. After the application of AquaGel with bacteria and organic compost with microorganisms, a greater number of spores was observed compared to the positive control in the third year of the experiment (2021: Control NPK – 2 pcs in 100 g of soil, AquaGel + Bacteria – 11 pcs in 100 g of soil). The use of microorganism-enriched hydrogels is increasingly explored. For example, wheat seeds treated with hydrogel-based bioinoculants together with the consortium of *Azotobacter chroococcum*, *Pseudomonas fluorescence*, and *Trichoderma viride* were beneficial to the growth of wheat plants (Suman et al. 2016). The combined use of the hydrogel consortium and the bacterial consortium *Planomicrobium chinense* and *Pseudomonas putida* was most effective in improving photosynthetic pigments, shoot fresh weight and soil fertility in soybean cultivation under drought conditions (Wagar et al. 2022). Research is also being conducted towards increasing the colonization of plant roots by using microbiologically enriched composts. The humus-rich compost significantly increased the growth of lettuce shoots, root growth and root colonization by AM fungi in the roots of the lettuce plants (Solaiman et al. 2019).

In the studies conducted, a negative effect of NPK fertilization on the colonization of apple tree roots by AM fungi and on the number of AM fungi spores in the soil was observed. The negative effect of long-term use of chemical fertilization on a significant reduction in the differentiation of AM fungi was observed in the soil in maize cultivation and in the rhizosphere of wheat (Liu et al. 2020; Ma et al. 2021; Jiang et al. 2021). Similarly, in the cultivation of strawberry 'Honeoye', the lowest values of the frequency and intensity of mycorrhizae were obtained after treatment with NPK (Sas-Paszt et al. 2015). Thus, the results obtained in this experiment confirm the negative effect of mineral fertilization on the colonization of plant roots by AM fungi.

The results obtained in this study will therefore contribute to obtaining more knowledge about the impact of bioproducts based on gel and compost enriched with beneficial microorganisms on the colonization of plant roots and soil by arbuscular mycorrhizal fungi.

CONCLUSIONS

The use of organic composts and beneficial soil microorganisms improves soil properties by supplying the soil with organic matter and increases microbiological diversity by increasing the presence of mycorrhizal fungi, which has a positive effect on plants, improving their growth and yield.

The use of hydrogels also has a positive effect on soil microbiota.

The results of the study indicate the most advantageous effect of compost combined with bacteria or fungi on the degree of root colonization by arbuscular mycorrhizal fungi and the formation of their spores in the soil.

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