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The influence of bioproducts on mycorrhizal occurrence in the vegetable roots

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Abstact: The aim of the study was to assess the impact of the use of biofertilizers on the degree of colonization of cucumber and tomato plant roots by arbuscular mycorrhizal fungi and the number of AMF spores in the rhizosphere soil. Two experiments were carried out in containers on cucumber and tomato plants under field conditions. The plants were fertilized with standard NPK fertilizer, mineral fertilizers: POLIFOSKA 6, Super FOS DAR 40 and urea in the full recommended dose and reduced by 40%, microbiologically enriched, and only with strains of beneficial microorganisms (*Bacillus* spp., *Bacillus amyloliquefaciens*, *Paenibacillus Polymyxa*, *Aspergillus niger*, *Purpureocillium lilacinum*). The experimental results showed a beneficial effect of the POLIFOSKA 6 mineral fertilizer enriched with beneficial bacteria of the Bacillus genus on increasing the colonization of the roots of tomato and cucumber plants by arbuscular mycorrhizal fungi. Compared to the control, fertilization with microbiologically enriched urea at doses of 100% and 60% reduced the frequency of mycorrhizas in the roots of tomato and cucumber plants. The use of POLIFOSKA 6 100% and Super FOS DAR 40 at a dose of 60% resulted in an increase in the number of spores in the rhizosphere soil. The experimental results will allow the development of new biofertilizers as alternative methods of fertilizing plants and improving soil quality compared to standard mineral fertilization.

Keywords: mycorrhizal fungi; spores; rhizosphere bacteria; tomato; cucumber; biofertilizers

In plant cultivation, it is very important to reduce the negative effects of biotic and abiotic environmental stresses in order to produce high-quality crops (Wu 2017). Biotic factors include stresses caused by pathogenic fungi, bacteria, nematodes, viruses, and pests, while abiotic factors include drought, mineral deficiency/excess, salinity, thermal stresses, and heavy metal contamination (Inculet et al. 2019). Among abiotic stresses, the most serious losses in horticultural and agricultural crops are caused by water shortage, especially long-term. Plants grown in field conditions are often exposed to the simultaneous effects of several stresses, a typical example of which is the occurrence of drought and high temperature, common in many agricultural areas not only in Poland but also throughout the world (Duc et al. 2018). Crop losses caused by stress factors can reach up to 50–82% (Inculet et al. 2019). These are the most significant threats to the cultivation and yielding of plants, and constitute serious limitations in plant production (Abewoy 2017). For this reason, a key role in plant production is played by sustainable plant cultivation technologies with the use of beneficial

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microorganisms, such as arbuscular mycorrhizal fungi (AMF) or rhizobacteria (PGPR). In many experiments, both field and container-based, AMF and PGPR have been shown to be beneficial in the cultivation of bean, corn, cucumber, and tomato plants (Krishnamoorthy et al. 2011). Beneficial bacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) play a key role in stimulating plant growth and yielding through the use of direct or indirect mechanisms such as: sharing mineral ions, production of phytohormones, inducing plant systemic resistance, or competing with harmful microorganisms for a soil niche (Inculet et al. 2019).

Mycorrhizal fungi play an important role in the mineral nutrition of plants, mainly in periods of drought and in soils with a low mineral content, because their mycelium hyphae can form a network many meters long, helping plants to take up and absorb minerals and water from the soil (Mannino et al. 2020). Through symbiotic interactions with plants, beneficial microorganisms play an important role in protecting plants against abiotic stresses such as nutrient deficiency (Volpe et al. 2018), high temperatures and drought (Wu 2017). Under stressful conditions, the presence of the mycelium of mycorrhizal fungi enables more intensive uptake of water and minerals by crop plants. Through their symbiotic interactions with plants, AM fungi have been found to increase induced tolerance to water shortage, including improved conductivity of stomata, increased water use efficiency, and reduced oxidative damage (Wu 2017). This is because arbuscular mycorrhizal fungi use hyphae to absorb and provide plants with available forms of N, P, K and micronutrients and water (Wang et al. 2014), and also use some mechanisms for combating diseases caused by soil pathogens (Widnyana et al. 2019).

The layer of soil adjacent to the surface of the roots, called the rhizosphere, inhabited by bacteria and fungi, is a site for the uptake of mineral ions; it is a zone where important physical, chemical and biological processes take place, of significance in the mineral nutrition of plants, their growth and yielding (Hashem et al. 2019). Bacteria referred to as rhizobacteria (PGPR), originating from the plant rhizosphere, have been shown to have positive effects on the growth of roots and above-ground parts of plants and their yielding (Hashem et al. 2019). The beneficial effects of rhizobacteria on the growth and yielding of plants are caused by direct mechanisms, including production of compounds that stimulate plant growth and alleviate environmental stress (Goswami et al. 2016). Species of the genus *Bacillus* belonging to PGPR form stress-resistant spores that can survive adverse environmental conditions such as drought, salinity, acidification, and high or low temperatures. These bacteria produce auxins and organic acids that stimulate plant growth and yielding, and also synthesize metabolites that are toxic to plant and soil pathogens, reducing their occurrence (Radhakrishan et al. 2017). Rhizosphere bacteria can be used in agricultural crops to alleviate biotic and abiotic stresses and to develop sustainable, environmentally friendly methods of plant fertilization (Mishra et al. 2017). The use of microorganisms in plant cultivation technologies is a new way to increase the tolerance of plants to abiotic stresses, especially to environmental stress caused by climate change. In modern agriculture, the use of beneficial rhizosphere bacteria, including *B. subtilis*, can limit the use of synthetic pesticides, fungicides and insecticides, as well as make it possible to take advantage of their positive effects in sustainable plant cultivation technologies and in organic farming (Wang et al. 2018).

An alternative to chemical means of production, which are used in excessive amounts in agriculture to obtain high yields, is the use of beneficial microorganisms as biofertilizers, biostimulants and biological plant protection products, which is a new approach in fertilization and plant cultivation technologies, and which is already intensively implemented in organic farming (Raklami et al. 2019). There are many biofertilizers available on the market that contain arbuscular mycorrhizal fungi or other microorganisms that promote plant growth and yielding, and improve soil fertility. Many of them have so far proved to be not very effective or ineffective, usually due to the low survival of microorganisms under unfavourable soil and climatic conditions, and their poor ability to colonize soil and plant roots (Figueiredo et al. 2011). Therefore, newly developed and marketed biopreparations for plant cultivation should be tested on various cultivars, under different plant cultivation conditions and under various climatic and environmental conditions, such as water availability, abundance of minerals in the soil and its physical structure and microbiological composition.

The aim of the study was to determine the influence of biofertilizers on mycorrhizal frequency of arbuscular mycorrhizal fungi in the roots of cucumber and tomato plants, and the number of spores of my-

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corrhizal fungi in rhizosphere soil under optimal (100%) and reduced (50%) plant irrigation.

MATERIAL AND METHODS

The experiments of this study were carried out in 2018 and 2019. The seeds of Zefir F_1 cucumber were purchased from the seed company PNOS Ożarów Mazowiecki (Poland), and were kept at 15 °C and 30% relative humidity until the initiation of the experiments. The seeds of Calista F_1 tomato were purchased from the seed company Hazera Poland Sp. z o.o. (Poland), and were kept at 15 °C and 30 % relative humidity until the initiation of the experiments.

Experiment I: Seedlings of Zefir F_1 cucumber and Calista F_1 tomato were planted in May in the Ecological Experimental Field of the Institute of Horticulture in Skierniewice. The cucumber plants were planted 60 cm × 100 cm apart, and the tomato plants 85 cm × 50 cm apart. The cucumber plots had an area of 8 m^2, while the tomato plots were 13.6 m^2. Plant care treatments were carried out in accordance with the recommendations for commercial field plantations. The experiment was conducted in a random block design with four replications, each consisting of a test plot with 12 cucumber plants and 32 tomato plants respectively.

All mineral fertilizers and beneficial microorganisms were scattered on the experimental plots and then mixed with the soil using available cultivation tools. The fertilization regimens presented in Table 1 were used in the experiment.

Experiment II: Seeds of Zefir F_1 cucumber were sown in May in large pots with a capacity of 35 L in the experimental field of the Warsaw University of Life Sciences in Skierniewice. Seedlings of Calista F_1 tomato were planted in May, 3 plants per pot, in the same experimental field. Plant care treatments were carried out in accordance with the requirements for commercial plantations. The experiment was designed in random blocks of four replications, each consisting of 3 pots with 3 plants. The plants were drip-irrigated. Each pot was irrigated with two CNL drop emitters (Netafim, Israel) with an output of 2 L/h. Irrigation was carried out automatically based on the measurement of soil moisture at a depth of 15 cm. The moisture measurements and valve operation were controlled with the Agreus system (Inventia, Poland).

Two levels of plant irrigation were used in the experiment:

– optimal irrigation to maintain soil moisture at 80 to 100% of field water capacity (FWC)

– limited irrigation, which was carried out when soil moisture decreased to a level of 40–50% FWC. In this combination, depending on the course of the weather, the plants were periodically subjected to water stress.

All mineral fertilizers and beneficial microorganisms were scattered on the experimental plots and then mixed with the soil using available cultivation tools. The fertilization regimens presented in Table 2 were used in the experiment.

The following analyses and observations were made:

– determination of root colonization by arbuscular mycorrhizal fungi,

– counting of the number of spores of mycorrhizal fungi in rhizosphere soil.

Determination of root colonization by arbuscular mycorrhizal fungi. The roots of tomato and cucumber (10 g from each replication), collected in July 2018 and 2019, were stained according to the method developed in the Department of Microbiology and Rhizosphere of The National Institute of Horticultural Research (Derkowska et al. 2015a). Next, 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 produced. The assessment of the degree of colonization of the roots by arbuscular mycorrhizal fungi was performed by the Trouvelot method (Trouvelot et al. 1986). Based on the results, mycorrhizal frequency (F%) was calculated using the computer program MYCOCALC, available from the website: http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/MYCOCALC.EXE (Tables 3–8).

Counting of the number of spores of mycorrhizal fungi in rhizosphere soil. Samples of rhizosphere soil, collected in July 2018 and 2019, were used to weigh out 100 g portions for further analyses. These were then placed in bottle containers and made up to 1 litre with distilled water. The resulting suspensions were shaken for approx. 1 hour and placed in a refrigerator for 24 h at 4 °C. After that, the soil solutions were filtered through a column of sieves (0.5 mm, 0.125 mm, 0.0063 mm, and 0.0045 mm). The fractions of soil remaining on the successive sieves were washed away with distilled water into Petri dishes

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Table 1. Experimental combinations used in the experiment I.

Treatment	Fertilization regimen	
	Cucumber plants (fertilizer dose in g/plot)	Tomato plants (fertilizer dose in g/plot)
1. Control NPK	POLIFOSKA 6 – 320 g, simple superphosphate – 42 g, potassium sulphate – 200 g, pre-sowing and ammonium nitrate used as top dressing in 2 doses – 290 g	POLIFOSKA 6 – 820 g, superphosphate – 60 g, pre-sowing and ammonium nitrate used as top dressing in 2 doses – 290 g
2. POLIFOSKA 6 at 100%	POLIFOSKA 6 – 550 g, potassium sulphate – 100 g, pre-sowing and ammonium nitrate used as top dressing in 2 doses – 290 g	POLIFOSKA 6 – 820 g, superphosphate – 60 g, pre-sowing and ammonium nitrate used as top dressing in 2 doses – 500 g
3. Urea 100%	Urea – 320 g, potassium sulphate – 120 g, ammonium nitrate – 100 g, pre-sowing and ammonium nitrate used as top dressing in 2 doses – 290 g	Urea – 540 g, potassium sulphate – 200 g, ammonium nitrate – 300 g, pre-sowing and ammonium nitrate used as top dressing in 2 doses – 370 g
4. Super FOS DAR 40 at 100%	Super FOS DAR 40 – 180 g, ammonium nitrate – 100 g, potassium sulphate – 310 g, pre-sowing and ammonium nitrate used as top dressing in 2 doses – 290 g	Super FOS DAR 40 – 310 g, ammonium nitrate – 100g, potassium sulphate – 520 g, pre-sowing and ammonium nitrate used as top dressing in 2 doses – 500 g
5. POLIFOSKA 6 at 100% + bacterial strains	POLIFOSKA 6 – 550 g, potassium sulphate – 100 g, pre-sowing and ammonium nitrate used for top dressing in two doses – 290 g *Bacillus* spp. *Bacillus amyloliquefaciens*, *Paenibacillus polymyxa* – 3.83 g	POLIFOSKA 6 – 820 g, superphosphate – 60 g, pre-sowing and ammonium nitrate used as top dressing in 2 doses – 500 g, *Bacillus* spp., *Bacillus amyloliquefaciens*, *Paenibacillus polymyxa* – 3.83 g
6. Urea 100% + fungal strains	Urea – 320 g, potassium sulphate – 120 g, ammonium nitrate – 100 g, pre-sowing and ammonium nitrate used as top dressing in 2 doses – 290 g, *Aspergillus niger*, *Purpureocillium lilacinum* – 5.25 g	Urea – 540 g, potassium sulphate – 200 g, ammonium nitrate – 300 g, pre-sowing and ammonium nitrate used as top dressing in 2 doses – 370 g, *Aspergillus niger*, *Purpureocillium lilacinum* – 5.25 g
7. Super FOS DAR 40 at 100% + bacterial strains	Super FOS DAR 40 – 180 g, ammonium nitrate – 100g, potassium sulphate – 310 g, pre-sowing and ammonium nitrate for top dressing in 2 doses – 290 g, *Bacillus* spp., *Bacillus amyloliquefaciens*, *Paenibacillus polymyxa* – 3.83 g	Super FOS DAR 40 – 310 g, ammonium nitrate – 100 g, potassium sulphate – 520 g, pre-sowing and ammonium nitrate for top dressing in 2 doses – 500 g, *Bacillus* spp., *Bacillus amyloliquefaciens*, *Paenibacillus polymyx*a – 3.83 g
8. Urea at 60% + fungal strains	Urea – 190 g, potassium sulphate – 70 g, ammonium nitrate – 60 g, pre-sowing and ammonium nitrate used as top dressing in 2 doses – 130 g, *Aspergillus niger*, *Purpureocillium lilacinum* – 5.25 g	Urea – 330 g, potassium sulphate – 120 g, ammonium nitrate – 100 g, pre-sowing and ammonium nitrate used as top dressing in 2 doses – 220 g, *Aspergillus niger*, Purpureocillium lilacinum – 5.25 g

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Table 1. to be continued

Treatment	Fertilization regimen	
	Cucumber plants (fertilizer dose in g/plot)	Tomato plants (fertilizer dose in g/plot)
9. POLIFOSKA 6 at 60% + bacterial strains	POLIFOSKA 6 – 330 g, potassium sulphate – 120 g top dressing in 2 doses ammonium nitrate – 180g, *Bacillus* spp., *Bacillus amyloliquefaciens*, *Paenibacillus polymyxa* – 3.83 g	POLIFOSKA 6 – 490 g, ammonium nitrate – 60 g, superphosphate – 30 g, pre-sowing and ammonium nitrate used as top dressing in 2 doses – 300 g, *Bacillus* spp., *Bacillus amyloliquefaciens*, *Paenibacillus polymyx*a – 3.83 g
10. Super FOS DAR 40 at 60% + bacterial strains	Super FOS DAR 40 – 110 g, ammonium nitrate – 40g, potassium sulphate – 180 g, pre-sowing and ammonium nitrate used as top dressing in 2 doses – 180 g, *Bacillus* spp., *Bacillus amyloliquefaciens*, *Paenibacillus polymyxa* – 3.83 g	Super FOS DAR 40 – 190 g, ammonium nitrate – 60 g, potassium sulphate – 310 g, pre-sowing and ammonium nitrate used as top dressing in 2 doses – 300 g, *Bacillus* spp., *Bacillus amyloliquefaciens*, *Paenibacillus polymyxa* – 3.83 g

(120 mm), to which sucrose (5 g per dish) was added. The thus prepared samples were examined using a Nikon SMZ 800 stereoscopic microscope, fishing out and counting spores of mycorrhizal fungi found in them (Błaszkowski 2008; Tables 3–8).

Characteristics of the applied mineral fertilizers. The applied mineral fertilizers as mineral components of the biofertilizers had the following characteristic features:

Urea (brand name: Pulrea®) – contains 46% nitrogen (N) in the amide form.

POLIFOSKA 6 (compound inorganic fertilizer NPK(S)) – in the form of granular fertilizer, containing 6% nitrogen (N) in the ammonium form, 20% phosphorus (P_2O_5), 30% potassium (K_2O) in the form of potassium salt, and 7% water-soluble sulfur trioxide (SO_3) in the form of sulphate.

Super FOS DAR 40 (inorganic fertilizer PSCa) – contains 40% P_2O_5 – phosphorus pentoxide, of which 25% P_2O_5 soluble in neutral citrate solution and water; 10% CaO – calcium oxide soluble in water, water-soluble S (as SO_3) – 5%, and microelements (Co, Cu, Fe, Mn, Zn), which are a valuable addition from natural phosphates, improving the absorption of other ingredients.

Characteristics of the microorganisms used

Aspergillus niger – these fungi synthesize phytohormones as one of the key mechanisms of plant growth stimulation and induce systemic resistance (ISR) in the plant, making it resistant to various diseases. Fungi of the species *Aspergillus* spp. produce siderophores, which make iron available to plants (Sayyed et al. 2013; Patel et al. 2016).

Purpureocillium lilacinum – this fungus synthesizes protease enzymes, keratinases, laminarases and chitinases. It also produces one of the most physiologically active auxins, indole acetic acid (IAA), and exhibits a number of antifungal properties that make *P. lilacinum* a suitable strain for the development of an organic biofertilizer (Cavello et al. 2015).

Bacillus spp. (*Bacillus amyloliquefaciens* and *Paenibacillus polymyxa*) – bacteria commonly found in soil. Their role is to accelerate the decomposition of organic compounds of plant origin, mainly carbohydrates and pectins, limit the growth of *Verticillium dahliae* by synthesizing pectino- and proteolytic enzymes, and to metabolize cellobiose. *Bacillus* spp. are also known to be antagonists especially against fungi such as *Fusarium*, *Rhizoctonia*, *Phytophthora*, and *Pythium*. They are also used as a plant growth stimulant (Radhakrishan et al. 2017; Hashem et al. 2019).

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.13.1 software (StatSoft Inc., 2011).

RESULTS

The results of the conducted experiments indicate a favourable influence of mineral fertilizers enriched with beneficial soil microorganisms and of the application of microorganisms on their own on the degree of root colonization by arbuscular mycorrhizal fungi

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Table 2. Experimental combinations used in the experiment II

Treatment	Fertilization regimen	
	Cucumber plants (fertilizer dose in g/plot)	Tomato plants (fertilizer dose in g/plot)
1. Control NPK	Soil fertilization 3 g of Super FOS DAR 40 granulated fertilizer, 12.5 g of potassium salt and 8.8 g of urea were used for each container in early spring in each year. Urea in the amount of 5 g per container was also used in mid-summer.	Soil fertilization 3 g of Super FOS DAR 40 granulated fertilizer, 12.5 g of potassium salt and 8.8 g of urea were used for each container in early spring in each year. Urea in the amount of 5 g per container was also used in mid-summer.
2. Control ZERO + fungal strains	No treatment mineral fertilizers. Beneficial soil fungi in the amount of 5.25 g per container were applied after planting the plants, thoroughly mixing them with the soil. The mixture of beneficial soil fungi contained two species: *Aspergillus niger* and *Purpureocillium lilacinum*.	No treatment mineral fertilizers. Beneficial soil fungi in the amount of 5.25 g per container were applied after planting the plants, thoroughly mixing them with the soil. The mixture of beneficial soil fungi contained two species: *Aspergillus niger* and *Purpureocillium lilacinum*.
3. Control ZERO + bacterial strains	No treatment mineral fertilizers, soil with beneficial bacteria. For each container, 3.83 g was applied along the rows immediately after planting the plants, thoroughly mixing them with the soil. The mixture of beneficial bacteria contained three strains of *Bacillus* (*Bacillus* sp., *Bacillus amyloliquefaciens* and *Paenibacillus polymyxa*).	No treatment mineral fertilizers, soil with beneficial bacteria. For each container, 3.83 g was applied along the rows immediately after planting the plants, thoroughly mixing them with the soil. The mixture of beneficial bacteria contained three strains of *Bacillus* (*Bacillus* sp., *Bacillus amyloliquefaciens* and *Paenibacillus polymyxa*).
4. Control NPK + fungal strains	Soil fertilization 3 g of Super FOS DAR 40 granulated fertilizer, 12.5 g of potassium salt and 8.8 g of urea were used for each container in early spring in each year. Urea in the amount of 5 g per container was also used in mid-summer. The mixture of beneficial soil fungi contained three strains of *Aspergillus niger*, *Purpureocillium lilacinum* – 5.25 g.	Soil fertilization 3 g of Super FOS DAR 40 granulated fertilizer, 12.5 g of potassium salt and 8.8 g of urea were used for each container in early spring in each year. Urea in the amount of 5 g per container was also used in mid-summer. The mixture of beneficial soil fungi contained three strains of *Aspergillus niger*, *Purpureocillium lilacinum* – 5.25 g.
5. Control NPK + bacterial strains	Soil fertilization 3 g of Super FOS DAR 40 granulated fertilizer, 12.5 g of potassium salt and 8.8 g of urea were used for each container in early spring in each year. Urea in the amount of 5 g per container was also used in mid-summer. The mixture of beneficial bacteria contained three strains of *Bacillus* (*Bacillus* sp., *Bacillus amyloliquefaciens* and *Paenibacillus polymyxa*) – 3.83 g.	Soil fertilization 3 g of Super FOS DAR 40 granulated fertilizer, 12.5 g of potassium salt and 8.8 g of urea were used for each container in early spring in each year. Urea in the amount of 5 g per container was also used in mid-summer. The mixture of beneficial bacteria contained three strains of *Bacillus* (*Bacillus* sp., *Bacillus amyloliquefaciens* and *Paenibacillus polymyxa*) – 3.83 g.
6. POLIFOSKA 6 at 100% + bacterial strains	POLIFOSKA 6 in a 100% dose with three strains of bacteria of the genus *Bacillus* (3.83 g), which had been incorporated into the fertilizer during the production of granules. It was applied at 6 g per container. Ammonium nitratre in the amount of 3 g was applied in spring, and subsequently in a dose of 2 g in mid-summer. Potassium potassium sulfate was given in the amount of 3 g.	POLIFOSKA 6 in a 100% dose with three strains of bacteria of the genus *Bacillus* (3.83 g), which had been incorporated into the fertilizer during the production of granules. It was applied at 10 g per container. Ammonium nitratre in the amount of 8 g was applied in spring, and subsequently in a dose of 5 g in mid-summer. Potassium potassium sulfate was given in the amount of 5 g.

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Table 2. to be continued

Treatment	Fertilization regimen	
	Cucumber plants (fertilizer dose in g/plot)	Tomato plants (fertilizer dose in g/plot)
7. Urea 100% + fungal strains	Urea in a 100% – 5 g, dose enriched with strains of filamentous fungi of the species *Aspergillus niger*, *Purpureocillium lilacinum* – 5.25 g. In addition, 3 g of potassium sulfate, 3 g of ammonium nitratre and 3 g of Super FOS DAR 40 fertilizer were added in spring to each container. Ammonium nitratre in the amount of 5 g was also applied in mid-summer.	Urea in a 100% – 8 g, dose enriched with strains of filamentous fungi of the species *Aspergillus niger*, *Purpureocillium lilacinum* – 5.25 g. In addition, 5 g of potassium sulfate, 3 g of ammonium nitratre and 5 g of Super FOS DAR 40 fertilizer were added in spring to each container. Ammonium nitratre in the amount of 5 g was also applied in mid-summer.
8. Super FOS DAR 40 at 100% + bacterial strains	Super FOS DAR 40 in a 100% dose enriched with Tyree strains of *Bacillus* bacteria in the amount of 3.83 g per container. In spring, the soil was fertilized with 3 g of potassium sulfate, Super FOS DAR 40 fertilizer in the amount of 3 g and ammonium nitratre – in early spring in the amount of 3 g, and in a dose of 5 g in the middle of summer.	Super FOS DAR 40 in a 100% – 8 g, dose enriched with three; strains of *Bacillus* bacteria in the amount of 3.83 g per container. In spring, the soil was fertilized with 5 g of potassium sulfate, Super FOS DAR 40 fertilizer in the amount of 5 g and ammonium nitratre – in early spring in the amount of 3 g, and in a dose of 5 g in the middle of summer.
9. Urea 60% + fungal strains	Urea in a 100% – 3 g, dose enriched with strains of filamentous fungi of the species *Aspergillus niger*, *Purpureocillium lilacinum* – 5.25 g. In addition, 3 g of potassium sulfate, 2 g of ammonium nitratre and 3 g of Super FOS DAR 40 fertilizer were added in spring to each container. Ammonium nitratre in the amount of 3 g was also applied in mid-summer.	Urea in a 100% – 5 g, dose enriched with strains of filamentous fungi of the species *Aspergillus niger*, *Purpureocillium lilacinum* – 5.25 g. In addition, 5 g of potassium sulfate, 2 g of ammonium nitratre and 5 g of Super FOS DAR 40 fertilizer were added in spring to each container. Ammonium nitratre in the amount of 3 g was also applied in mid-summer.
10. POLIFOSKA 6 at 60% + bacterial strains	POLIFOSKA 6 in a 100% dose with three strains of bacteria of the genus *Bacillus* (3.83 g), which had been incorporated into the fertilizer during the production of granules. It was applied at 4 g per container. Ammonium nitratre in the amount of 3 g was applied in spring, and subsequently in a dose of 2 g in mid-summer. Potassium potassium sulfate was given in the amount of 3 g.	POLIFOSKA 6 in a 100% dose with three strains of bacteria of the genus *Bacillus* (3.83 g), which had been incorporated into the fertilizer during the production of granules. It was applied at 6 g per container. Ammonium nitratre in the amount of 5 g was applied in spring, and subsequently in a dose of 5 g in mid-summer. Potassium potassium sulfate was given in the amount of 5 g.
11. Super FOS DAR 40 at 60% + bacterial strains	Super FOS DAR 40 in a 100% dose enriched with three strains of *Bacillus* bacteria in the amount of 3.83 g per container. In spring, the soil was fertilized with 2 g of potassium sulfate, Super FOS DAR 40 fertilizer in the amount of 3 g and ammonium nitratre – in early spring in the amount of 3 g, and in a dose of 5 g in the middle of summer.	Super FOS DAR 40 in a 100% – 8 g, dose enriched with Tyree strains of *Bacillus* bacteria in the amount of 3.83 g per container. In spring, the soil was fertilized with 3 g of potassium sulfate, Super FOS DAR 40 fertilizer in the amount of 5 g and ammonium nitratre – in early spring in the amount of 3 g, and in a dose of 5 g in the middle of summer.

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Table 3. Effect of mineral fertilization enriched with beneficial soil microorganisms on the degree of colonization of the roots of Zefir F_1 cucumber by arbuscular mycorrhizal fungi (IO Ecological experimental field, 2018–2019)

Treatment	Mycorrhizal frequency (*F*, %)	Relative mycorrhizal intensity (*M*, %)	Absolute mycorrhizal intensity (*m*, %)	Spore count (per 100 g soil)
1. Control NPK	10.55^{a}	0.53^{a}	5.99^{a}	10^{a}
2. POLIFOSKA 6 at 100%	30.0^{bc}	1.91^{ab}	5.30^{a}	15.5$^{a–c}$
3. Urea 100%	18.89^{ab}	1.49^{ab}	5.10^{a}	12^{ab}
4. Super FOS DAR 40 at 100%	26.67^{a-c}	2.39^{ab}	7.24^{a}	21$^{b–d}$
5. POLIFOSKA 6 at 100% + bacterial strains	42.78^{c}	3.23^{b}	7.34^{a}	31^{cd}
6. Urea 100% + fungal strains	21.67^{ab}	2.18^{ab}	7.09^{a}	14^{ab}
7. Super FOS DAR 40 at 100% + bacterial strains	29.45^{bc}	2.23^{ab}	7.07^{a}	26.5$^{c–e}$
8. Urea 60% + fungal strains	14.45^{ab}	0.97^{ab}	5.74^{a}	12^{ab}
9. POLIFOSKA 6 at 60% + bacterial strains	21.67^{ab}	1.90^{ab}	6.50^{a}	25.5$^{c–e}$
10. Super FOS DAR 40 at 60% + bacterial strains	26.11$^{a–c}$	1.99^{ab}	6.78^{a}	32.5^{d}

$^{a–c}$Means in columns marked with the same letter do not differ significantly at $P = 0.05$ according to Tukey's multiple test

(AMF) and the number of AMF spores in the soil, compared to the roots and soil of control plants fertilized only with mineral fertilizers (NPK). The method of plant cultivation (field cultivation and pot experiment) had no effect on the results of the experiments. The imposed water deficit increased the colonization of the roots of the studied vegetable plant species by mycorrhizal fungi (AMF) (Figures 1 and 2, Tables 5–8). By comparison, cucumber plants growing in the experimental combinations: POLIFOSKA 6 100% enriched with bacterial strains and Urea 60% enriched with fungal strains, in optimal water conditions, were characterized by the most extensive colonization of roots by mycorrhizal fungi. Plants from these experimental combinations were more frequently colonized by mycorrhizal fungi compared to plants growing under water deficit (Figures 1 and 2, Tables 5–8).

The use of the POLIFOSKA 6 mineral fertilizer in the full dose recommended by the manufacturer, enriched with beneficial bacteria of the genus *Bacillus* significantly increased mycorrhizal frequency in the roots of tomato and cucumber plants by arbuscular mycorrhizal fungi (Tables 3–8). The cultivation method (field or pot) of tomato and cucumber, with the same fertilization with biofertilizers, had no significant effect on the degree of root colonization by arbuscular mycorrhizal fungi (AMF) (Tables 3–8). Fertilization with microbiologically enriched urea in doses of 100% and 60% reduced mycorrhizal frequency in the roots of tomato and cucumber plants,

Table 4. Effect of mineral fertilization enriched with beneficial soil microorganisms on the degree of colonization of the roots of Calista F_1 tomato by arbuscular mycorrhizal fungi (IO Ecological experimental field, 2018–2019)

Treatment	Mycorrhizal frequency (*F*, %)	Relative mycorrhizal intensity (*M*, %)	Absolute mycorrhizal intensity (*m*, %)	Spore count (per 100 g soil)
1. Control NPK	10.56^{a}	1.04^{a}	8.88^{bc}	5^{a}
2. POLIFOSKA 6 at 100%	32.78^{de}	2.19^{bc}	6.75^{ab}	16^{bc}
3. Urea 100%	17.78$^{a–c}$	1.73$^{a–c}$	9.93^{c}	7.5^{ab}
4. Super FOS DAR 40 at 100%	28.89$^{c–e}$	2.14^{bc}	7.39$^{a–c}$	16.5^{bc}
5. POLIFOSKA 6 at 100% + bacterial strains	38.34^{e}	2.34^{c}	6.13^{a}	33.5^{d}
6. Urea 100% + fungal strains	21.11$^{a–c}$	1.70$^{a–c}$	7.98$^{a–c}$	18^{bc}
7. Super FOS DAR 40 at 100% + bacterial strains	28.34$^{c–e}$	2.46^{c}	8.72$^{a–c}$	13^{ab}
8. Urea 60% + fungal strains	16.12^{ab}	1.35^{ab}	8.46$^{a–c}$	12.5^{ab}
9. POLIFOSKA 6 at 60% + bacterial strains	27.78$^{c–e}$	2.43^{c}	8.76$^{a–c}$	25^{cd}
10. Super FOS DAR 40 at 60% + bacterial strains	24.45^{bd}	1.91$^{a–c}$	7.76$^{a–c}$	26.5^{cd}

$^{a–c}$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 5. Effect of the applied water regimen (reduction by 50%) and mineral fertilization enriched with beneficial soil microorganisms on the degree of colonization of the roots of Zefir F_1 cucumber by arbuscular mycorrhizal fungi (pot experiment, SGGW field in Skierniewice, 2018–2019)

Treatment	Mycorrhizal frequency (*F*, %)	Relative mycorrhizal intensity (*M*, %)	Absolute mycorrhizal intensity (*m*, %)	Spore count (per 100 g soil)
1. Control NPK	9.45^{a}	0.81^{a}	7.75^{a}	6^{a}
2. Control ZERO + fungal strains	16.11^{ab}	1.45^{ab}	6.44^{a}	14^{a}
3. Control ZERO + bacterial strains	28.89^{ab}	2.36^{ab}	8.13^{a}	17^{ab}
4. Control NPK + fungal strains	15.56^{ab}	1.10^{ab}	7.22^{a}	6.5^{a}
5. Control NPK + bacterial strains	26.67^{ab}	2.10^{ab}	6.18^{a}	14^{a}
6. POLIFOSKA 6 at 100% + bacterial strains	41.67^{b}	3.40^{b}	8.88^{a}	29^{b}
7. Urea 100% + fungal strains	17.22^{ab}	1.38^{ab}	7.38^{a}	8^{a}
8. Super FOS DAR 40 at 100% + bacterial strains	26.11^{ab}	1.89^{ab}	6.47^{a}	17.5^{ab}
9. Urea 60% + fungal strains	17.78^{ab}	1.66^{ab}	6.29^{a}	10^{a}
10. POLIFOSKA 6 at 60% + bacterial strains	21.67^{ab}	2.72^{ab}	7.84^{a}	19^{ab}
11. Super FOS DAR 40 at 60% + bacterial strains	22.23^{ab}	2.17^{ab}	7.0^{a}	18^{ab}

$^{a–c}$Means in columns marked with the same letter do not differ significantly at $P = 0.05$ according to Tukey's multiple test

compared to the control and plants fertilized with POLIFOSKA 6 biofertilizer (including beneficial bacteria of the genus *Bacillus*) (Tables 3–8). The application of fungi belonging to the species *Aspergillus niger* and *Purpureocillium lilacinum* reduced the colonization of the roots of tomato and cucumber plants by arbuscular mycorrhizal fungi, compared to the other fertilization combinations (Tables 3–8).

Similar results were obtained when comparing the relative mycorrhizal intensity (*M*, %). The application of beneficial bacteria and the POLIFOSKA 6 mineral biofertilizer in the full (100%) dose increased the intensity of root colonization of tomato and cucumber plants by arbuscular mycorrhizal fungi (Tables 3–8). Fertilization with urea microbiologically enriched with *Aspergillus niger* and *Purpureocillium lilacinum* reduced the relative intensity of root colonization of the studied plant species by AMF mycorrhizal fungi, compared to the fertilization with POLIFOSKA 6 enriched with *Bacillus* spp. bacteria (Tables 3–8). The results of the absolute mycorrhizal intensity (*m*, %) are not as unequivocal. In the field experiment with cucumber plants and the pot experiment with tomato and cucumber plants irrigated

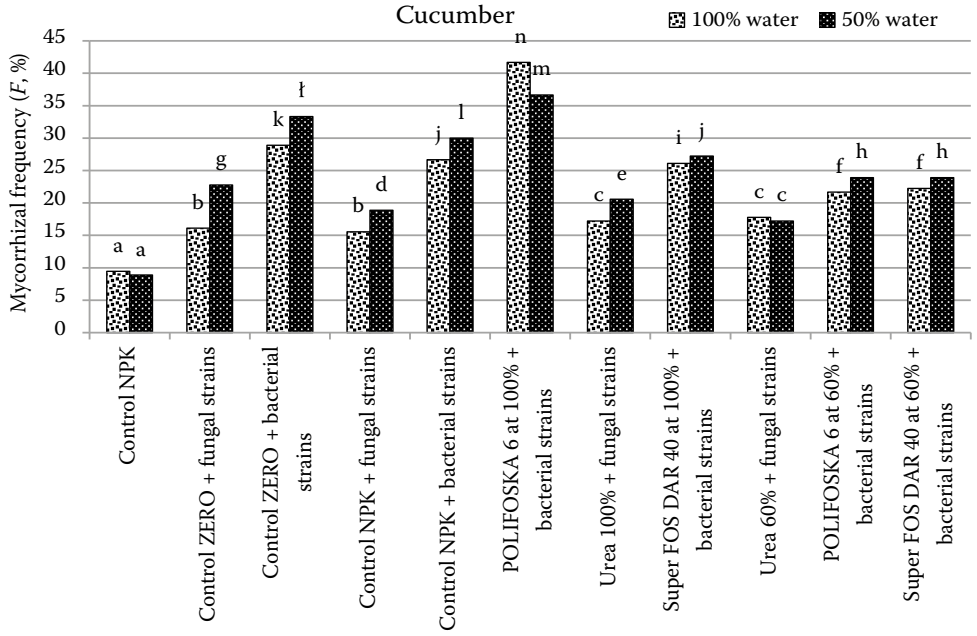

Figure 1. Effect of mineral fertilization and biofertilization on mycorrhizal frequency (*F*, %) in the roots of tomato plants growing under optimal and 50% reduced water conditions (SGGW, 2018–2019)

$^{a–n}$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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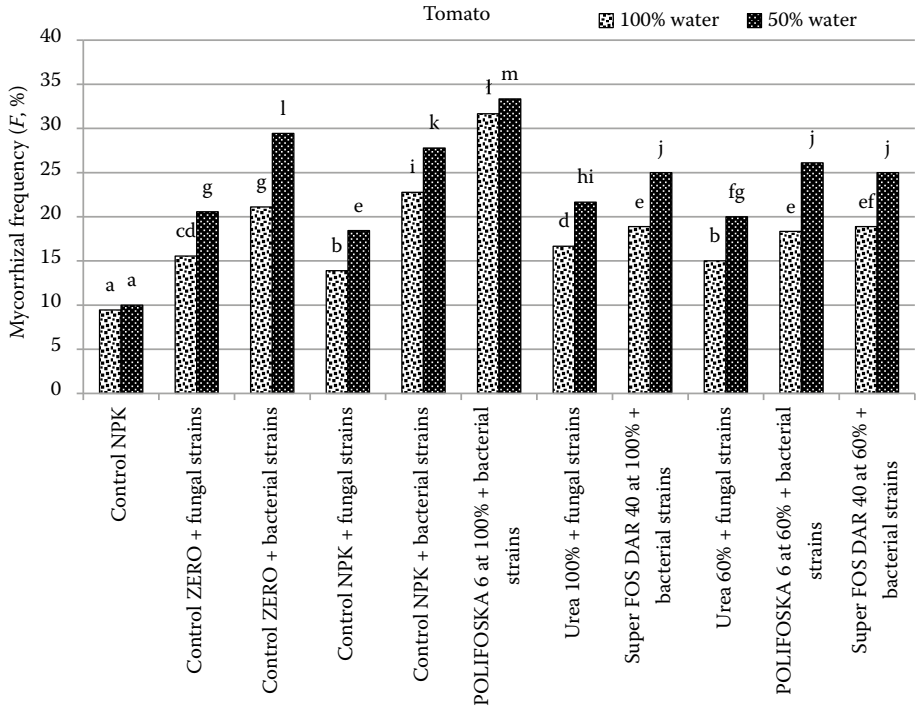

Figure 2. Effect of mineral fertilization and biofertilization on mycorrhizal frequency (*F*, %) in the roots of cucumber plants growing under optimal and 50% reduced water conditions (SGGW, 2018–2019)

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

with a 50% dose of water, the absolute mycorrhizal intensity decreased as a result of the application of NPK and microbiologically enriched urea, and was similar in terms of mycorrhizal frequency (*F*, %) and relative mycorrhizal intensity (*M*, %) (Tables 3–8). In the remaining experimental variants, both the application of beneficial fungi and of bacteria, as well as NPK fertilization of tomato and cucumber plants increased the absolute mycorrhizal intensity (*m*, %) (Tables 3–8).

The results of the study indicate favourable effects of the biofertilizers and their microbiologically enriched variants on the number of spores of mycorrhizal fungi in the rhizosphere soil. Regardless of the cultivation method (field cultivation or pot experiment), the use of microbiologically enriched POLIFOSKA 6 in a dose of 100%, increased the number of spores in the rhizosphere soil under periodically occurring soil drought conditions, i.e. reduced doses of irriga-

Table 6. Effect of the applied water regimen and mineral fertilization enriched with beneficial soil microorganisms on the degree of colonization of the roots of Zefir F_1 cucumber by arbuscular mycorrhizal fungi (pot experiment, SGGW field in Skierniewice, 2018–2019)

Treatment	Mycorrhizal frequency (*F*, %)	Relative mycorrhizal intensity (*M*, %)	Absolute mycorrhizal intensity (*m*, %)	Spore count (per 100 g soil)
1. Control NPK	8.89[a]	0.85[a]	8.67[ab]	5.5[a]
2. Control ZERO + fungal strains	22.78[a–d]	2.31[ab]	10.13[b]	11[ab]
3. Control ZERO + bacterial strains	33.33[cd]	2.78[ab]	8.31[ab]	18[bc]
4. Control NPK + fungal strains	18.89[ab]	1.45[ab]	7.67[ab]	10[ab]
5. Control NPK + bacterial strains	30.0[b–d]	2.24[ab]	7.31[a]	17[bc]
6. POLIFOSKA 6 at 100% + bacterial strains	36.67[d]	3.13[b]	8.47[ab]	28[c]
7. Urea 100% + fungal strains	20.56[a–c]	1.63[ab]	7.80[ab]	11.5[ab]
8. Super FOS DAR 40 at 100% + bacterial strains	27.[23b–d]	2.47[ab]	8.51[ab]	18.5[bc]
9. Urea 60% + fungal strains	17.22[ab]	1.41[ab]	8.37[ab]	10[ab]
10. POLIFOSKA 6 at 60% + bacterial strains	23.89[b–d]	2.32[ab]	9.45[ab]	20[bc]
11. Super FOS DAR 40 at 60% + bacterial strains	23.89[b–d]	1.84[ab]	7.74[ab]	23[c]

[a–c]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 7. Effect of the applied water regimen (reduction by 50%) and mineral fertilization enriched with beneficial soil microorganisms on the degree of colonization of the roots of Calista F_1 tomato by arbuscular mycorrhizal fungi (pot experiment, SGGW field in Skierniewice, 2018–2019)

Treatment	Mycorrhizal frequency (*F*, %)	Relative mycorrhizal intensity (*M*, %)	Absolute mycorrhizal intensity (*m*, %)	Spore count (per 100 g soil)
1. Control NPK	9.45^{a}	0.72^{a}	6.93^{a}	5^{a}
2. Control ZERO + fungal strains	15.56^{ab}	1.49^{a}	7.98^{a}	8^{ab}
3. Control ZERO + bacterial strains	21.11^{ab}	1.65^{a}	8.32^{a}	13.5^{ab}
4. Control NPK + fungal strains	13.89^{ab}	0.96^{a}	6.55^{a}	11.5^{ab}
5. Control NPK + bacterial strains	22.78^{ab}	1.85^{a}	8.04^{a}	14^{ab}
6. POLIFOSKA 6 at 100% + bacterial strains	31.67^{b}	2.69^{a}	7.84^{a}	29.5^{c}
7. Urea 100% + fungal strains	16.67^{ab}	1.38^{a}	8.17^{a}	13^{ab}
8. Super FOS DAR 40 at 100% + bacterial strains	18.89^{ab}	1.40^{a}	6.95^{a}	19.5^{bc}
9. Urea 60% + fungal strains	15.0^{ab}	1.02^{a}	5.20	11.5^{ab}
10. POLIFOSKA 6 at 60% + bacterial strains	18.34^{ab}	1.28^{a}	7.17^{a}	18.5^{bc}
11. Super FOS DAR 40 at 60% + bacterial strains	18.89^{ab}	1.42^{a}	6.66^{a}	14.4^{ab}

$^{a–c}$Means in columns marked with the same letter do not differ significantly at $P = 0.05$ according to Tukey's multiple test

tion water. The exception was the application of microbiologically enriched Super FOS DAR 40 in a dose of 60%, which increased the number of AMF spores in the rhizosphere soil in cucumber field cultivation.

DISCUSSION

The use of mineral fertilizers, agents improving soil fertility and biostimulants usually increases their effectiveness, improves the uptake of minerals from the soil and contributes to the increase in soil activity or has an antagonistic/negative effect on crop plants and soil microorganisms (Rouphael et al. 2015). However, the use of large doses of mineral fertilizers may reduce the population of beneficial microorganisms in the soil, mainly mycorrhizal fungi (Derkowska et al. 2015b, 2017). Unlike mineral fertilization, the long-term use of biofertilizers and organic fertilizers increases the population

Table 8. Effect of the applied water regimen and mineral fertilization enriched with beneficial soil microorganisms on the degree of colonization of the roots of Calista F_1 tomato by arbuscular mycorrhizal fungi (pot experiment, SGGW field in Skierniewice, 2018–2019).

Treatment	Mycorrhizal frequency (*F*, %)	Relative mycorrhizal intensity (*M*, %)	Absolute mycorrhizal intensity (*m*, %)	Spore count (per 100 g soil)
1. Control NPK	10.0^{a}	1.05^{a}	10.04^{b}	6^{a}
2. Control ZERO + fungal strains	20.56^{ab}	2.11^{b}	10.17^{b}	10^{ab}
3. Control ZERO + bacterial strains	29.44^{b}	2.35^{b}	8.11^{ab}	16^{ab}
4. Control NPK + fungal strains	18.45^{ab}	1.55^{ab}	7.86^{ab}	11^{ab}
5. Control NPK + bacterial strains	27.78^{b}	2.41^{b}	8.70^{ab}	15.5^{ab}
6. POLIFOSKA 6 at 100% + bacterial strains	33.34^{b}	2.44^{b}	7.36^{a}	26.5^{b}
7. Urea 100% + fungal strains	21.67^{ab}	1.87^{ab}	8.51^{ab}	15^{ab}
8. Super FOS DAR 40 at 100% + bacterial strains	25.0^{ab}	1.85^{ab}	7.39^{a}	15.5^{ab}
9. Urea 60% + fungal strains	20.0^{ab}	1.76^{ab}	8.79^{ab}	9.5^{ab}
10. POLIFOSKA 6 at 60% + bacterial strains	26.11^{ab}	2.46^{b}	9.46^{ab}	15.5^{ab}
11. Super FOS DAR 40 at 60% + bacterial strains	25.0^{ab}	2.05^{b}	8.15^{ab}	16.5^{ab}

$^{a–c}$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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of beneficial microorganisms and improves the biological activity of the soil, mainly by supplying it with organic carbon (Wu 2017). Song et al. (2015) in their long-term studies showed that organic fertilizers rich in nutrients, with a high content of organic C and total N in the soil, were characterized by a lower number of arbuscular mycorrhizal fungi (AMF) in the soil and roots. The results of the observations carried out in the presented experiments are consistent with the results of the cited authors. Fertilization with urea and urea enriched with beneficial microorganisms limited the colonization of roots and soil by mycorrhizal fungi. Other own research also indicates an increase in the population of arbuscular mycorrhizal fungi in the roots of plants growing in conditions of phosphorus deficiency in the soil. The deficiency of P, N, K, Mg and microelements in the soil stimulates the formation of symbiotic relationships between plant roots and mycorrhizal fungi, which particularly increases the availability of phosphorus (P) ions and other minerals for plants via hyphae (Sas-Paszt et al. 2019). The size of the AMF population and the effectiveness of their symbiosis with plants may also be influenced by bacteria living in the soil, associated with the soil environment itself or with plant roots (Rouphael et al. 2015). Research by Kameoka et al. (2019) indicate that the germination of AMF spores is induced by fatty acids and other lipid compounds secreted by bacteria into the soil environment. Similar results were obtained in the experiments of our study. Both the roots of tomato and cucumber plants and the surrounding soil were more often colonized by arbuscular mycorrhizal fungi after the application of a consortium of microorganisms (*Bacillus* spp., *Bacillus amyloliquefaciens* and *Paenibacillus polymyxa*) with mineral fertilizers compared to the application of mineral fertilizers alone and control plants fertilized with NPK (Tables 3–8). It is well known that in horticultural crops, including fruit trees, vegetable plants (tomato, cucumber) and ornamental plants, the presence of arbuscular mycorrhizal fungi in the soil and roots increases the tolerance of plants to drought and other environmental stresses (Baum et al. 2015) and favorably affects the size of the root system by increasing the length, density, diameter and number of lateral roots (Wu 2017). Moreover, arbuscular mycorrhizal fungi (AMF) not only enable increased water uptake by the plant's root system, but also stimulate it to use water better and more efficiently (Omirou et al. 2013). Research by Subramanian et al. (2006) showed that inoculation of tomato seedlings (*Solanum lycopersicum* L.) with the arbuscular mycorrhizal fungus Rhizophagus intraradices increased root colonization by AMF by up to 48%. Similar results were obtained by the authors of this study. Compared to control tomato plants, all water stress treatments increased root colonization by arbuscular mycorrhizal fungi (Figures 1 and 2, Tables 3–8). After fertilization with POLIFOSKA 6 100% fertilizers enriched with bacterial strains and Ureka 60% enriched with fungal strains, more colonization by arbuscular mycorrhizal fungi was observed in the roots of plants growing in optimal water conditions (AMF, Figure 1, Tables 5–8). In the remaining experimental combinations, the roots of cucumber plants growing in water deficit conditions were abundantly inhabited by mycorrhizal fungi (Figure 2, Tables 5–8). These results are consistent with the results of research by other authors on the stimulating effect of drought stress on the increasing colonization of plant roots by arbuscular mycorrhizal fungi.

CONCLUSIONS

The results of the experiments showed favourable effects of the mineral fertilizers enriched with beneficial soil microorganisms and the application of microorganisms on their own on increasing the degree of root colonization by arbuscular mycorrhizal fungi and the presence of AMF spores in the soil, compared to the roots and soil of control plants. The conducted research indicates beneficial effects of the use of biofertilizers as an effective alternative to intensive mineral fertilization and limiting the negative effects of soil fatigue and drought stress. The obtained results will allow the development of new biofertilizers as alternative methods of fertilizing plants and improving soil quality compared to standard mineral fertilization.

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