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Influence of beneficial soil microorganisms and mineral fertilizers enriched with them on the flowering, fruiting, and physical and chemical parameters of the fruit of three-year-old strawberry plants in field cultivation

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Abstract: The excessive use of chemicals in plant production, including mineral fertilizers has a harmful effect on the morpho- and physiological state of strawberry plants, their yielding, and the physicochemical properties of the fruit and soil. Increasing the effectiveness of beneficial microorganisms, i.e. filamentous fungi, arbuscular mycorrhizal fungi (AMF) and bacteria, is an essential method of reducing the amount of fertilizers used to fertilize the soil. The aim of the study was to assess the effect of beneficial soil microorganisms (filamentous fungi and bacteria) applied alone or together with mineral fertilizers on the morpho- and physiological state of 'Marmolada' strawberry plants, their yielding, and the physico-chemical properties of the fruit. The experiment included the application of fungi (*Aspergillus niger* and *Purpureocillium lilacinum*), bacteria *(Bacillus* sp., *Bacillus amyloliquefaciens* and *Paenibacillus polymyxa*) alone or together with mineral fertilizers (Polifoska 6, Urea, Super Fos Dar 40). The study investigated the number of inflorescences and flowers, the intensity of the green colour of leaves, fruit yield and weight (g and %), pH, extract (°Brix), titratable acidity (g/100 g), fruit firmness (N), as well as the concentrations of macro-and microelements in the leaves of the plants. The obtained results depend on the type of fertilizer and its concentration. Strains of filamentous fungi or bacterial strains only (without fertilizer), increase the fruit yield by 60 and 35%, respectively. Reduced to 60% doses of Polifoska 6, Urea, and Super Fos Dar 40 combined with beneficial soil microorganisms influence the strawberry fruit yield more beneficially than the fertilizers applied in standard doses (100%). The mineral fertilizers enriched with soil beneficial fungi and bacteria increase the mean fruit weight by 25–30%. Both Super Fos Dar 40 enriched with bacterial strains and Polifoska 6 at 100% positively affect the physical and chemical properties of strawberry fruits. The application of reduced to 60% doses of Fos Dar 40 and Polifoska 6 enriched with beneficial bacterial strains increase the nitrogen and potassium contents in the leaves, respectively. Urea and Super Fos Dar 40 at 100% enriched with beneficial bacteria positively affect magnesium content in the leaves. Application of Polifoska 6 and NPK enriched with bacteria beneficially influences the iron content in the leaves. The calcium content in strawberry leaves decreases due to fertilization.

Keywords: fertilization; beneficial microorganisms; fruit yield; fruit quality; *Fragaria* × *ananassa*

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Numerous research works emphasize the harmfulness of the excessive use of chemicals in plant production, including mineral fertilizers, and the need to replace them with products of organic origin that are safer for the environment and human health (Pylak et al. 2019; Sarkadi 2019). One of the methods of reducing the amount of fertilizers used to fertilize the soil is to increase their effectiveness by making use of beneficial microorganisms, such as filamentous fungi, arbuscular mycorrhizal fungi (AMF), and also bacteria, which are responsible for improving the growth and physiological condition of plants. These microorganisms are also recommended for the protection of plants against diseases and pests (Sas-Paszt et al. 2019a, b, 2020a, b; Canassa et al. 2020; Karnwal, Kapoor 2021). There is a belief among producers of agricultural crops that the use of high doses of mineral fertilizers not only harms the environment, but also unnecessarily increases plant production costs and causes eutrophication of surface and ground waters, degradation and pollution of the soil environment, drinking water and air (Boy, Arcand 2013). An alternative to this is the implementation of modern plant cultivation technologies based on natural raw materials for the production of bio-fertilizers and soil improvers (Sas-Paszt, Głuszek 2019; Grzyb et al. 2012a,b; Malusa et al. 2013; Derkowska et al. 2015; Sas-Paszt, Głuszek 2019; Sas-Paszt et al. 2019a, b). The results of many studies from recent years indicate that the use of fertilizers enriched with beneficial strains of bacteria and fungi increases the effectiveness of fertilizers used in plant cultivation (Derkowska et al. 2015; Sas-Paszt et al. 2019a, b; Delaporte-Quintana et al. 2020; Sas-Paszt et al. 2020a, b). Soil microorganisms strengthen the physiological condition of plants and their resistance to biotic and abiotic environmental stresses (Kumar et al. 2019b; Abbasi et al. 2020; Bilal et al. 2020; Liu et al. 2020). Research studies have proven that the beneficial bacteria of the genus *Bacillus*, producing compounds that provide phosphorus to plants, the auxin IAA and other plant growth regulators such as cytokinins, have a beneficial effect on plant growth and yielding (Zerrouk et al. 2020; de Sousa et al. 2021). Benefits of the use of Bacilli and other microbes on strawberry growth and yielding have been observed in other trials (Sas-Paszt et al. 2019a, b; Vicente-Hernández et al. 2019; Mei et al. 2021).

In agriculture, not only on small farms but also on large-scale farms, environmentally friendly fertilizers are being sought to replace the increasingly scarce manure and composts of plant origin and at the same time to reduce the negative effects of intensive use of artificial mineral fertilisers. One innovative solution deserving special attention is the enrichment of mineral and natural fertilizers, composts, innovative organic fertilizers and various liquid preparations with beneficial microorganisms. These can be either single strains or consortia of them (Ravnskov et al. 2006; Sas-Paszt et al. 2015, 2019a, b, 2020a, b). The use of native filamentous fungi and beneficial bacteria strains in new bioproducts introduced on the market will provide plants with more favourable living conditions and will promote better plant nutrition and keep the soil healthy. Taking advantage of these microorganisms in agriculture can lead to a significant reduction in the use of high doses of chemicals, commonly considered harmful to the environment (Derkowska et al. 2013; Lingua et al. 2013; Grzyb et al. 2015a, b; Sas-Paszt et al. 2011, 2019a, b).

In the issues of fertilization efficiency and environmental protection, much attention has recently been paid to the root system of plants, especially its morphological features, which are modified in the soil by various abiotic and biotic factors (Fan et al. 2011; Sas-Paszt et al. 2019a, b). The studies have shown that beneficial soil microorganisms also mitigate the impact of adverse environmental factors acting on the plant (Sas-Paszt et al. 2011; Ojuederie et al. 2019; Sas-Paszt et al. 2019a, b, 2020a, b).

There have been very few results of scientific experiments evaluating the effect of microbiologically enriched mineral fertilizers on the yielding of strawberry plants. There are also few results available of studies on the effectiveness of the application to the soil of beneficial microorganisms without additional mineral fertilization in the cultivation of plant species of high economic importance. The development of new, environmentally friendly technologies requires further research on the use of microbiologically enriched mineral fertilizers and the impact of such treatments on the growth and yielding of strawberry plants, and the quality of strawberry fruit.

The aim of the study is to assess the effect of beneficial soil microorganisms used on their own without additional mineral fertilization, as well as of innovative and traditionally used mineral fertilizers enriched with bacteria and filamentous fungi on the fruiting of aging three-year-old strawberry plants growing in the open field.

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Table 1. Parameters of the soil in the experiment

pH (KCl)	Humus content	Macroelements		Microelements						
		P	K	Mg	B	Cu	Fe	Mn	Na	Zn
		(mg/100 g)		(mg/1 000 g)						
6.2	1.2%	7.5	12.4	5.8	2.4	4.8	862	75.5	4.35	3.7

MATERIAL AND METHODS

Experimental conditions. The experiment was established in the spring of 2018 in the Experimental Orchard of The National Institute of Horticultural Research in Dąbrowice (Central Poland, 51°91'41''N, 20°11'13''E, 145 m a.s.l.) and will run for three consecutive years. The presented data in this paper were obtained in 2020. The subject of the research are strawberry plants of the cultivar 'Marmolada' (synonym 'Onebor'). Frigo A+ plantlets (15–18 mm) were planted in early May on a podzolic soil underlaid by sandy loam, rated as soil quality class 3b. Soil mineral content at the experimental site is shown in Table 1.

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

On a single plot of 3 m in length, 12 plants were planted. The distances between the plants in a row were 0.25 m and between the rows 1 m. The experiment was established in a random block design in three replications. Each experimental combination was represented by 36 plants.

During the growing season, the weeds on the experimental plots were removed by hand. Irrigation of the plants was carried out on the basis of tensiometers using an automatically controlled dripline. The microbiologically enriched fertilizers and microorganisms applied to the soil on their own were repeated every year (Table 2). The growth of strawberry plants on the basis of the number of inflorescences and flowers, as well as fruit yield, fruit quality and the physical and chemical properties of the fruit, was assessed. The intensity of the green colour of leaves was assessed in mid-July, and the concentrations of macro- and microelements at the end of August.

Evaluation of the flowering intensity of strawberry plants and the intensity of the green colour of leaves. For the plants from each plot, the number of inflorescences and flowers on them was determined three times every 5 days in late May/

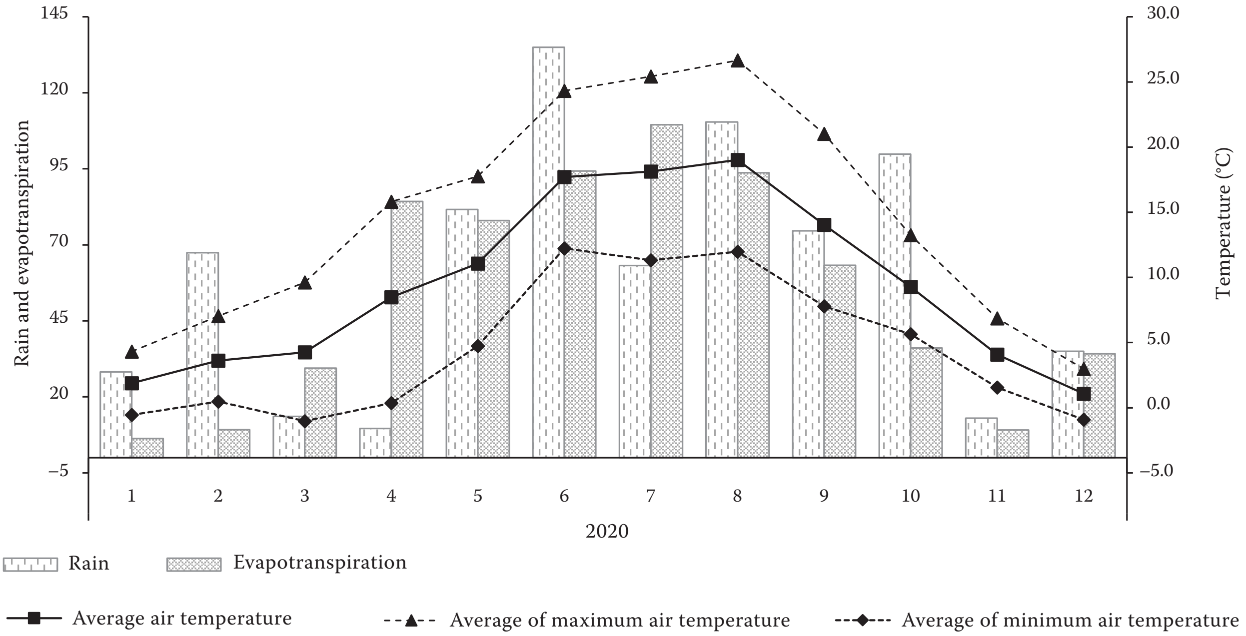

Figure 1. Thermal and humidity conditions at the experimental site (data from the weather station located in the experimental orchard

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early June. The average results for each fertilization combination are given as the numbers counted. The intensity of the green colour of the leaves was determined using a SPAD 502 meter (Konica Minolta, Japan)with a sample of 5 fully expanded leaves taken from two plants in each replication. The measurements were recorded between the base and apex of each leaf blade in the middle of July. During measurements with the meter, the sensor head was shaded with the operator's own body as recommended by the manufacturer to avoid direct sunlight from reaching the instrument.

Assessment of fruit yield and mean fruit weight. Strawberry fruits from each plot were harvested four times at intervals of 4–5 days. This period covered the high-quality commercial yield. The following parameters were determined:

– number of fruits from five representative plants in each replication, expressed as the numbers counted,
– weight of fruits (g),
– average weight of one fruit (g) calculated by dividing the total weight of fruits by the number of fruits.

The fruit yield (g/plant), the fruit yield (% compared to control plants) and fruit weight (g), fruit weight (% compared to control plants) were registered on the same plants on which the number of inflorescences and flowers were previously determined.

Determination of physico-chemical parameters of strawberry fruits. The chemical parameters analyses were performed one week after the first

Table 2. The experimental combination included in the experiment

No.	Treatment	Fertilization applied
1	Control (no treatment)	Unfertilized podzolic soil (mineral composition given above).
2	NPK	Standard soil fertilization: 12 g of Super Fos Dar 40 granulated fertilizer, 50 g of potassium salt and 35 g of Urea were used on an individual plot. Urea in the amount of 12 g per plot was also applied in mid-summer.
3	Fungal strains	Beneficial soil fungi only, in the amount of 5.25 g per plot were applied along the rows, thoroughly mixing them with the soil. The mixture of beneficial soil fungi contained two species: *Aspergillus niger* and *Purpureocillium lilacinum.*
4	Bacterial strains	Beneficial bacteria only, applied along the rows for each plot, 3.83 g, thoroughly mixing them with the soil. The mixture of beneficial bacteria contained three strains of *Bacillus* (*Bacillus* sp., *Bacillus amyloliquefaciens* and *Paenibacillus polymyxa*).
5	NPK + fungal strains	Fertilization as in point 2 with the beneficial soil fungi listed in point 3.
6	NPK + bacterial strains	Fertilization as in point 2 and the beneficial bacteria applied to the soil as in point 4.
7	Polifoska 6 100% + bacterial strains	Fertilizer 26 g, including three strains of bacteria of the genera *Bacillus* and *Paenibacillus*, 3.83 g. Urea, in the amount of 30 g , and the second time in the amount of 20 g in mid-summer; potassium salt was used in the amount of 33 g.
8	Urea 100% + fungal strains	Enriched with strains of filamentous fungi of the species and quantitative composition as in point 3. For each plot, 50 g of potassium salt, 35 g of Urea, in spring, 20 g was also applied in mid-summer, and 12 g of Super Fos Dar 40 fertilizer was used.
9	Polifoska 6 100%	Fertilizer 26 g , 50 g of potassium salt, and 30 g of Urea were used per one plot. Urea in the amount of 20 g was also used in mid-summer.
10	Super Fos Dar 40 100% + bacterial strains	Enriched with three strains of *Bacillus* and *Paenibacillus* bacteria was applied in the amount of 3.83 g per plot. In spring, with 50 g of potassium salt, 12 g of Super Fos Dar 40, Urea – 35 g and 20 g in mid-summer.
11	Urea 60% + fungal strains	Enriched with strains of filamentous fungi of the species and quantitative composition as in point 3. In spring, for each plot – 30 g of potassium salt, 20 g of Urea and 12 g of Super Fos Dar 40 were used. Urea in the amount of 12 g was also applied in mid-summer.
12	Polifoska 6 60% + bacterial strains	Enriched with three strains of bacteria of the genera *Bacillus* and *Paenibacillus* was used in the same way as in point 7. 14 g of Polifoska 6, 30 g of potassium salt and 18 g of Urea were used for each plot. Urea in the amount of 12 g was also used in mid-summer.
13	Super Fos Dar 40 60% + bacterial strains	Enriched with three *Bacillus* and *Paenibacillus* bacterial strains was used in the same way as in point 7. 7 g of Super Fos Dar 40, 50 g of potassium salt and 20 g of Urea were used per plot. Urea in the amount of 12 g was also used in mid-summer.

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harvest. The refractometric extract (soluble-solids content) was determined using a Mettler Toledo type RE50 refractometer (Mettler Toledo, Switzerland). The measurements were made in duplicate for an average sample obtained after grinding the fruit. The results were expressed in °Brix. Titratable acidity was determined in accordance with PN-90/A-75101/04 using a Mettler Toledo type DL 58 titrator (Mettler Toledo, , Switzerland). The measurements were performed in duplicate for an average sample obtained after grinding the fruit. The results were expressed in g/100 g in terms of citric acid. Active acidity (pH) was measured with a Mettler Toledo type DL 58 titrator. The measurements were made in duplicate on an average sample obtained after grinding the fruit. Fruit firmness was expressed as the force needed to pierce the skin of the fruit with a 3.2 mm diameter flat plunger moving at a speed of 50 mm/min. The measurements were made using an Instron 4303 testing machine (Instron Ltd., High Wycombe, England) equipped with a 10 N measuring head. The measurements were performed on a sample of 15 fruits on the side surface at the level of the largest diameter. The results were expressed in Newtons (N).

Analysis of mineral elements in strawberry leaves. Nitrogen (N) content was determined according to Dumas, PB-05; edition 02. Concentrations of phosphorus (P), potassium (K), magnesium (M), and calcium (Ca) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES), PB-01. Concentrations of boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) were determined by inductively coupled plasma optical emission spectrometry ICP-OES; PB-10. Dry (absolute) weight (DW) was determined using the gravimetric method: PB-O9.

Statistical analysis. The results were statistically analyzed using one-way analysis of variance with the Duncan test, α = 0.05, using the statistical program Statistica (Ver. 13.1, 2017) Data not significantly different from each other are marked with the same letters.

RESULTS

The field study on three-year-old strawberry plants showed that the type and method of fertilization had no significant effect on the number of inflorescences and flowers produced, nor on the chlorophyll content in the leaves of this species (Figures 2, 3, 4). Only in the plants fertilized with Urea in a 100% dose enriched with filamentous fungi there was a tendency to increase the number of flowers (Figure 3).

The fruiting of the strawberry plants in the control combination without fertilization had a low value. Supplying those plants with the NPK fertilizer in the spring improved their yielding (Figure 5). Filamentous fungi added to the mineral fertilizers

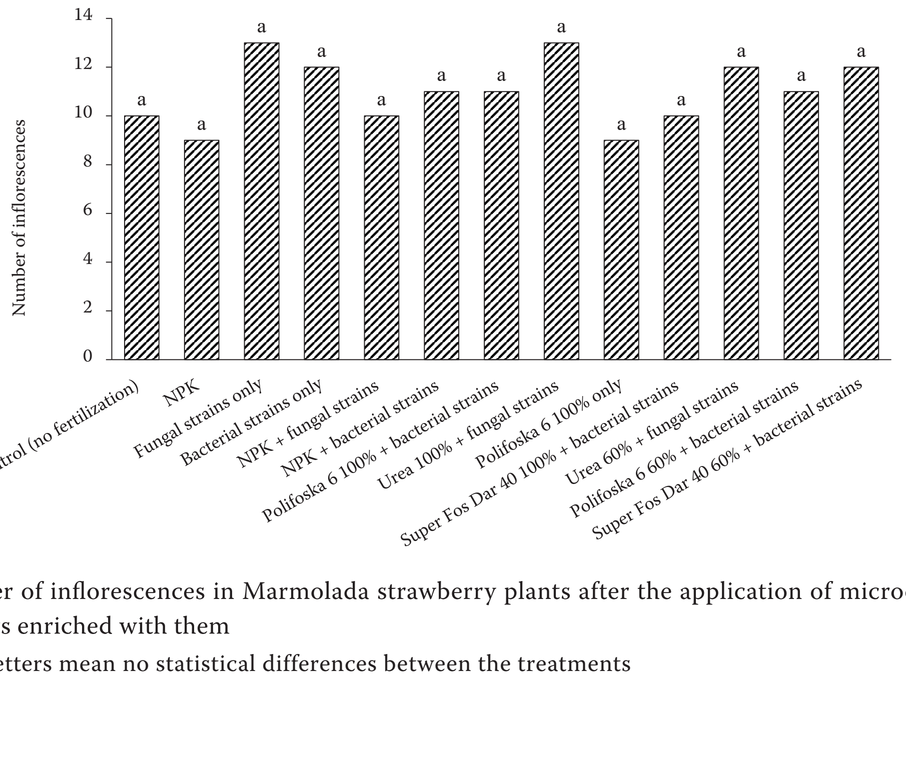

Figure 2. Number of inflorescences in Marmolada strawberry plants after the application of microorganisms and mineral fertilizers enriched with them

The same small letters mean no statistical differences between the treatments

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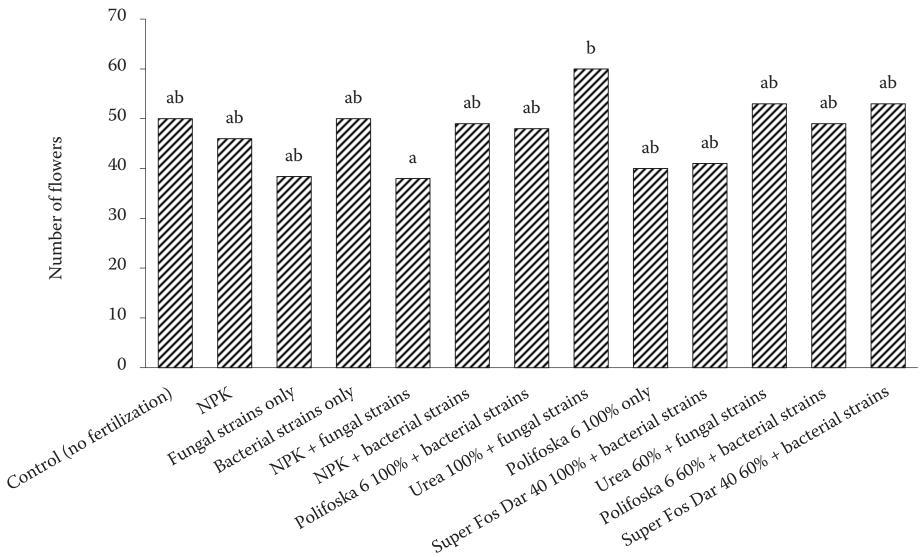

Figure 3. Number of flowers in Marmolada strawberry plants after the application of microorganisms and mineral fertilizers enriched with them

Different small letters mean statistical differences between the treatments

significantly increased the fruiting potential of the plants, but to a different extent depending on the type of fertilizer and its concentration. Reduced doses of the Polifoska 6 and Super FosDar 40 fertilizers in combination with microorganisms had a more favourable effect on fruiting than the fertilizers in the combinations where the doses of the mineral fertilizers used for fertilizing the plants were higher by almost 50%.

The increases in fruit yield, in percentage terms, ranged from 20 to 72% depending on the applied fertilizers and microorganisms (Figure 6). Low yields were found following the use of microbiologically enriched (with filamentous fungi) NPK,

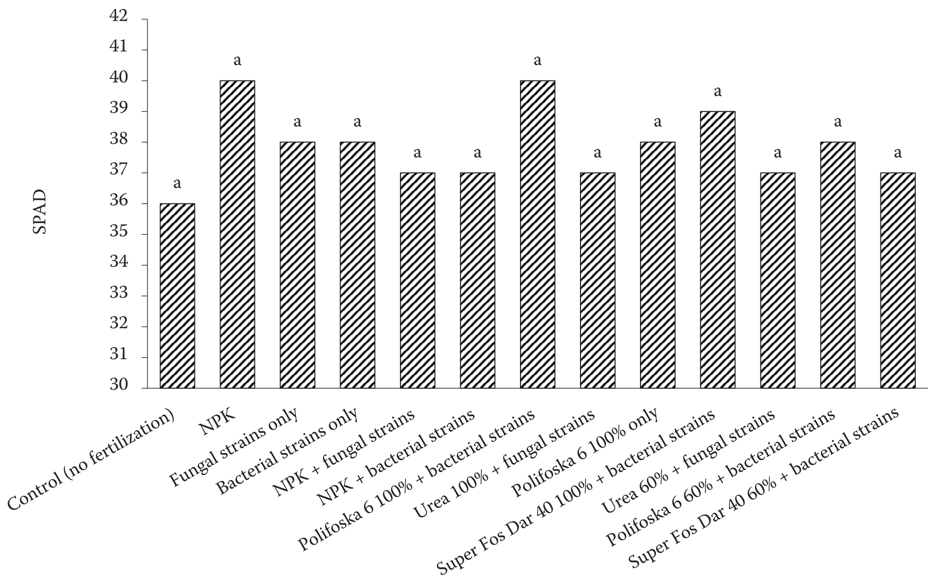

Figure 4. The green colour intensity (SPAD) of the leaves of Marmolada strawberry plants after the application of microorganisms and mineral fertilizers enriched with them

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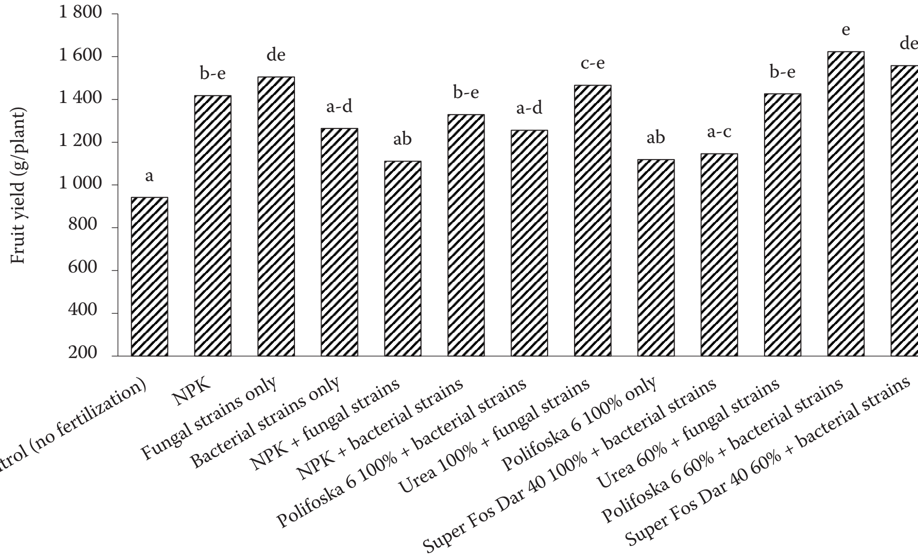

Figure 5. Fruit yield of Marmolada strawberry plants after the application of microorganisms and mineral fertilizers enriched with them

Different small letters mean statistical differences between the treatments

Polifoska 6 at 100% without microorganisms, and Super Fos Dar 40 at 100% enriched with bacteria. On the other hand, in the group of fertilizers strongly stimulating the yielding of strawberry plants there were filamentous fungi on their own and Urea enriched microbiologically in both concentrations, while in the group with the highest productivity index – fertilizers used at 60%, such as Urea enriched with fungi, and microbiologically enriched Polifoska 6 and Super Fos Dar 40.

Single strawberry fruits from the non-fertilized control plots, with the exception of the NPK combination, had a lower weight than those from the plots fertilized with the mineral fertilizers enriched with beneficial microorganisms, i.e. bacteria of the genus *Bacillus* and filamentous fungi: *Aspergillus*

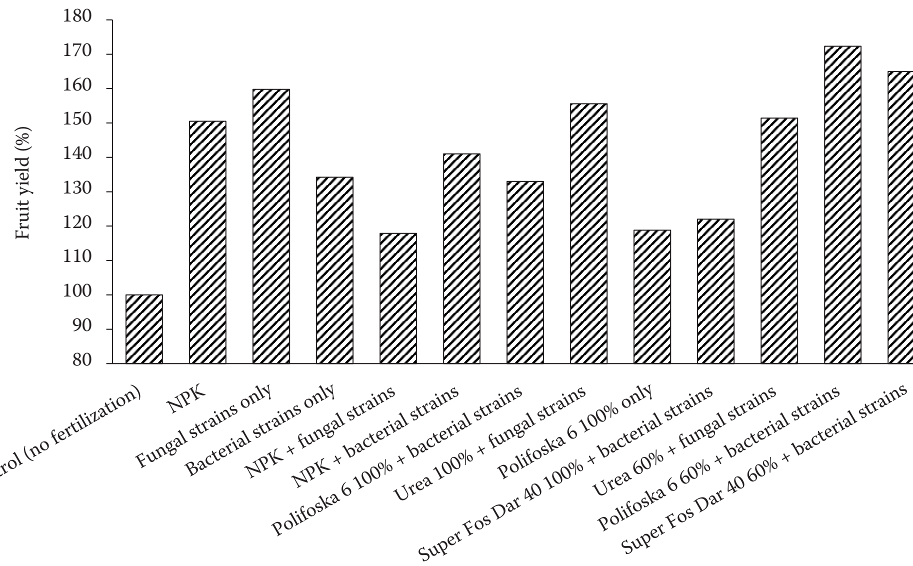

Figure 6. Fruit yield of Marmolada strawberry plants after the application of microorganisms and mineral fertilizers enriched with them

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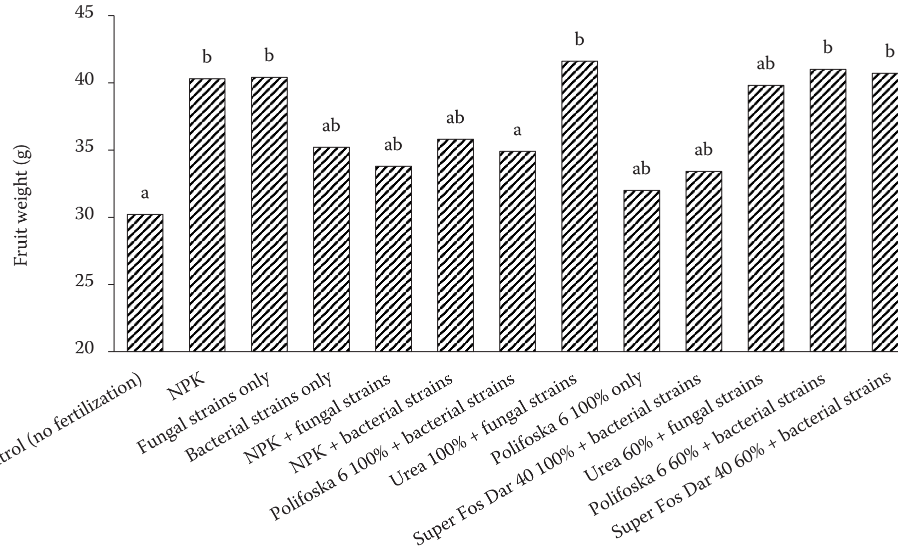

Figure 7. Fruit weight of Marmolada strawberry plants after the application of microorganisms and mineral fertilizers enriched with them

Different small letters mean no statistical differences between the treatments

niger and *Purpureocillium lilacinum* (Figure 7). Because of their presence, the increase in the weight of one fruit, especially in the case of reduced doses of some fertilizers, ranged from 10–12 grams. Urea at 100% enriched with filamentous fungi also had a positive effect on the increase in fruit weight.

As a result of fertilization with microorganisms alone, the mean fruit weight increased from 16.6 to 33.7% (Figure 8). A high increase in fruit weight, from 33.4 to 37.7%, occurred under the influence of Urea used both at 100% and 60% enriched with fungi, and also the fertilizers Polifoska 6 and Super Fos Dar 40 used at a conc. of 60% enriched with bacterial strains. By comparison, the use of Polifoska 6 at a conc. of 100%, enriched with bacteria and without their presence, resulted

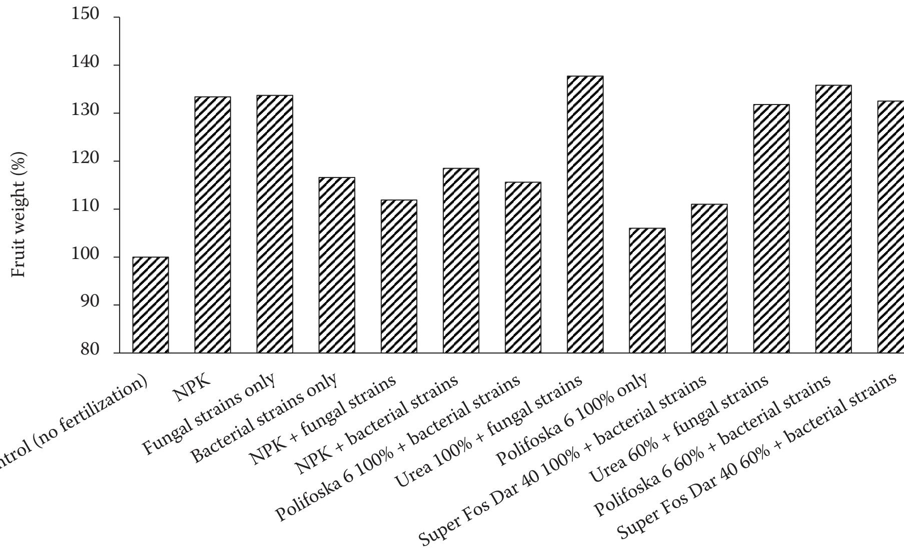

Figure 8. Fruit weight of Marmolada strawberry plants after the application of microorganisms and mineral fertilizers enriched with them

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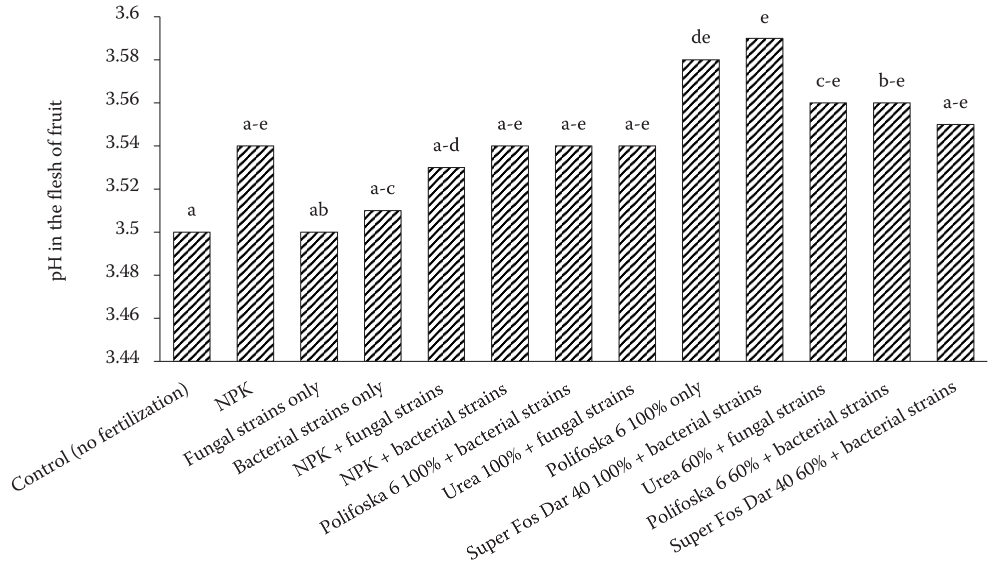

Figure 9. The pH of Marmolada strawberry fruits after the application of microorganisms and mineral fertilizers enriched with them

Different small letters mean no statistical differences between the treatments

in only a slight increase in fruit weight, ranging from 6 to 15%. A significant increase in fruit weight (about 33%) was caused by the NPK fertilizer on its own, without the support of microorganisms.

The method in which strawberry plants were fertilized had no significant effect on the physicochemical properties of the fruit. All the measurements of extract, titratable acidity and fruit firmness in individual combinations were within the limits of the statistical error (Figures 9–12).

Only the pH of the fruit flesh increased significantly under the influence of the Polifoska 6 fertilizer, as well as the Super Fos Dar 40 fertilizer in a dose of 100% enriched with strains of bacte-

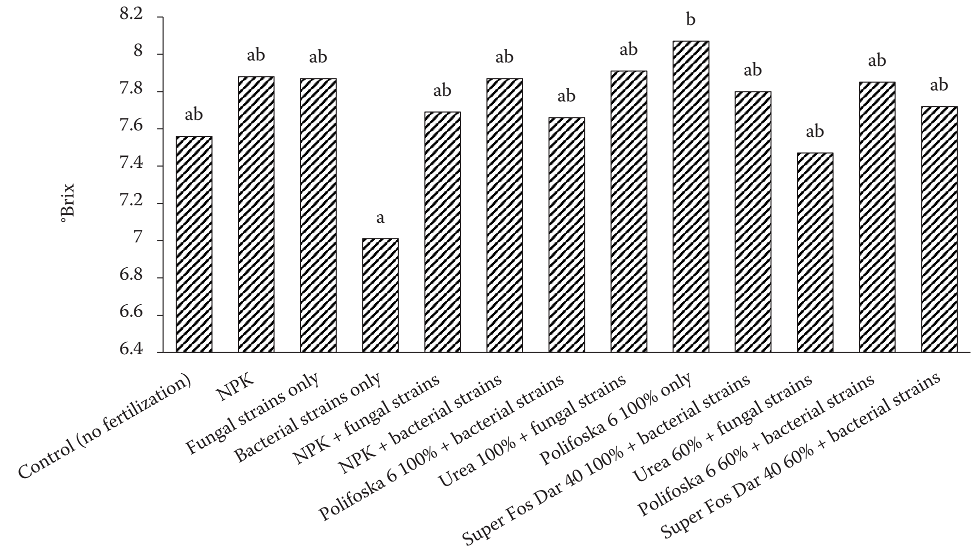

Figure 10. The soluble solids content (extract) of Marmolada strawberry fruits after the application of microorganisms and mineral fertilizers enriched with them

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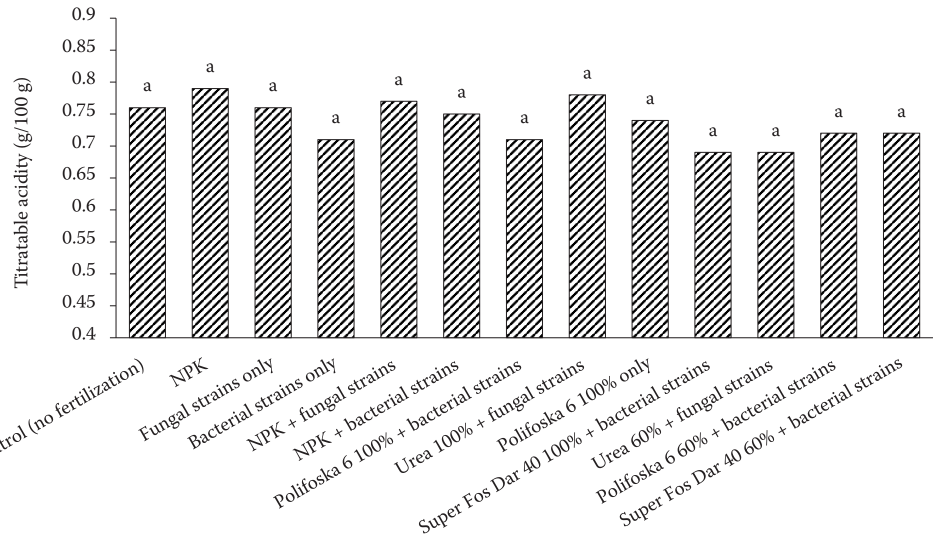

Figure 11. The titratable acidity of Marmolada strawberry fruits after the application of microorganisms and mineral fertilizers enriched with them

The same small letters mean no statistical differences between the treatments

ria of the genus *Bacillus*. Urea at a conc. of 60% enriched with filamentous fungi and Polifoska 6 at a concentration of 60% enriched with bacteria also caused a significant increase in pH.

The chemical analyses showed that the effect of the type of fertilization on the macroelements content in strawberry leaves was in most cases insignificant (Table 3). The nitrogen level increased significantly when the plants were fertilized with Super Fos Dar 40 at 60% enriched with beneficial bacterial strains. In the case of phosphorus, the type of fertilization had no effect on the level of this component in the leaves. The potassium content increased when the plants were fertilized with Poli-

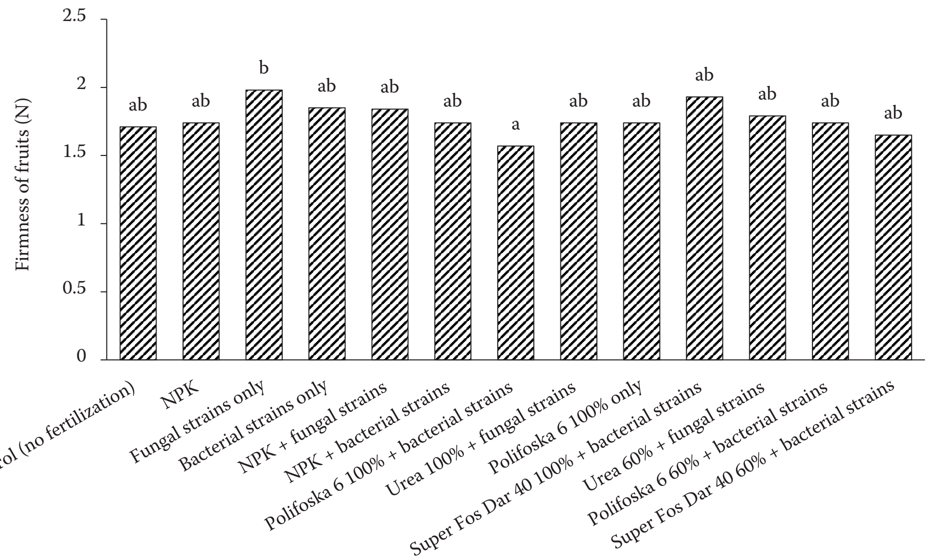

Figure 12. The firmness of Marmolada strawberry fruits after the application of microorganisms and mineral fertilizers enriched with them

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foska 6 at 60% enriched with bacteria. A significant increase in the level of magnesium was found following the application of such fertilizers as Urea and Super Fos Dar 40 at 100% enriched with beneficial bacteria. The tendency to increase the level of this component in the leaves was also observed after fertilizing with NPK enriched with either filamentous fungi or beneficial bacteria. The calcium level in the leaves decreased under the influence of all the applied fertilizers, regardless of their modification.

The type and method of strawberry fertilization did not, except in very few cases, have a significant effect on the concentrations of microelements in the leaves (Table 4). The only exception was iron. Its level increased mainly under the influence of Polifoska 6 at 100% without bacteria and at 60% when enriched with bacteria. A similar increase in the level of iron was caused by applying the NPK fertilizer enriched with bacteria.

DISCUSSION

The necessity to protect the environment and the continually growing awareness of the need for chemically uncontaminated agricultural production compel us to verify the methods used so far to increase the production of agricultural crops by reducing the doses of artificial fertilizers and plant protection products used against diseases and pests. Numerous studies conducted around the world indicate that the synthetic fertilizers and chemicals used in agricultural production that are harmful to the environment can be partially reduced by replacing them with products of organic origin (Pylak et al. 2019). Therefore, attempts are being made to use beneficial microorganisms for this purpose. Increases in the effectiveness of fertilizers in stimulating plant growth and improving their fruiting, and even replacement of mineral fertilization can be achieved by using, among others, beneficial microorganisms such as bacteria and fungi. Like soil improvers, they have a positive effect on the physiological condition of plants, stimulate their growth and yielding, improve soil quality, and also protect plants against diseases and pests (Sas-Paszt et al. 2019a, b; Trzciński et al. 2021).

Research results obtained by some authors indicate that the treatment of plants with beneficial microorganisms increases the total plant biomass, number of flowers, chlorophyll content and fruit yield and quality in strawberry (Todeschini et al. 2018; Şahin et al. 2019; Morais et al. 2019). De Andrade et al. (2019) observed that combined application of a 50% dose of a mineral fertilizer with beneficial bacteria promotes the growth of aboveground part of strawberry plants in comparison with non-fertilized treatments, whereas root growth is improved in the combination without fertilizer. Combined application of bacteria with

Table 3. Effect of microbiologically enriched mineral fertilizers on the concentrations of macronutrients in the leaves of Marmolada strawberry plants (The National Institute of Horticultural Research, Experimental Orchard, Dąbrowice, 2020)

Combination (treatment)		N	P	K	Mg	Ca
		(% DW)				
1	Control (no fertilization)	1.65^{ab}	0.18^{ab}	1.22^{ab}	0.26^{bc}	1.50^{d}
2	Control NPK	1.76^{b-d}	0.19^{ab}	1.42^{cd}	0.25^{bc}	1.43^{cd}
3	Fungal strains only	1.59^{a}	0.18^{ab}	1.25^{a-c}	0.23^{a}	1.38^{bc}
4	Bacterial strains only	1.60^{a}	0.17^{a}	1.32^{b-d}	0.24^{ab}	1.40^{bc}
5	NPK + fungal strains	1.73^{b-d}	0.19^{ab}	1.24^{a-c}	0.27^{cd}	1.33^{a-c}
6	NPK + bacterial strains	1.83^{d}	0.20^{ab}	1.35^{b-d}	0.27^{cd}	1.31^{a-c}
7	Polifoska 6 100% innovative fertilizer + bacterial strains	1.71^{bc}	0.19^{ab}	1.28^{a-c}	0.26^{bc}	1.30^{a-c}
8	Urea 100% + fungal strains	1.76^{b-d}	0.20^{ab}	1.25^{a-c}	0.28^{d}	1.28^{ab}
9	Polifoska 6 100% only	1.65^{ab}	0.20^{ab}	1.20^{ab}	0.27^{cd}	1.22^{a}
10	Super Fos Dar 40 100% + bacterial strains	1.74^{b-d}	0.20^{ab}	1.10^{a}	0.28^{d}	1.37^{bc}
11	Urea 60% + fungal strains	1.71^{bc}	0.19^{ab}	1.28^{a-c}	0.25^{bc}	1.43^{cd}
12	Polifoska 6 60% + bacterial strains	1.95^{e}	0.22^{b}	1.49^{d}	0.26^{bc}	1.36^{a-d}
13	Super Fos Dar 40 60% + bacterial strains	1.79^{cd}	0.16^{a}	1.27^{a-c}	0.26^{bc}	1.41^{bc}

Different small letters mean statistical differences between the treatments

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Table 4. Effect of microbiologically enriched mineral fertilizers on the concentrations of microelements in the leaves of Marmolada strawberry plants

Combination (treatment)		B	Cu	Fe	Mn	Zn
		(mg/kg DW)				
1	Control (no fertilization)	42.0[bc]	3.25[a]	150[a]	104[a]	19.3[a]
2	Control NPK	40.2[bc]	3.13[a]	161[ab]	163[b]	17.0[a]
3	Fungal strains only	40.8[bc]	3.10[a]	182[a–c]	146[ab]	16.1[a]
4	Bacterial strains only	43.3[c]	3.12[a]	219[a–d]	112[ab]	18.9[a]
5	NPK + fungal strains	32.4[a–c]	2.98[a]	190[a–c]	122[ab]	20.9[a]
6	NPK + bacterial strains	34.4[a–c]	3.37[a]	247[cd]	109[ab]	20.1[a]
7	Polifoska 6 100% innovative fertilizer + bacterial strains	33.2[a–c]	2.98[a]	214[a–d]	126[ab]	18.0[a]
8	Urea 100% + fungal strains	30.6[ab]	2.90[a]	214[a–d]	139[ab]	19.1[a]
9	Polifoska 6 100%	38.9[a–c]	4.10[a]	261[d]	133[ab]	27.1[a]
10	Super Fos Dar 40 100% + bacterial strains	27.7[a]	3.28[a]	206[a–d]	85.8[a]	24.4[a]
11	Urea 60% + fungal strains	35.3[a–c]	3.06[a]	169[ab]	91.5[ab]	19.8[a]
12	Polifoska 6 60% + bacterial strains	37.7[a–c]	3.93[a]	228[b–d]	119[ab]	27.0[a]
13	Super Fos Dar 40 60% + bacterial strains	41.1[bc]	3.07[a]	162[ab]	156[ab]	23.5[a]

Different small letters mean statistical differences between the treatments

a 50% dose of fertilizer also increases the total dry weight of strawberry plants. The results of our research on chlorophyll are consistent with the data of Sas-Paszt et al. (2019a, b).

Srivastav et al. (2019) report that in the combinations with strawberry plants fertilized with microorganisms or microbiologically enriched organic fertilizers, a greater number of flowers per one plant was obtained, flowering accelerated, and the number of fruits and the percentage fruit-set increased. Moreover, the fruit harvesting period was longer than in the control combination. In our own research on three-year-old strawberry plants, we found no such correlations with the type of fertilization as those reported by the above-mentioned authors in their work. The type of fertilization in three-year-old strawberry plants had no significant effect on the number of inflorescences or flowers. However, it is worth mentioning that in two-year-old plants in the same experiment, such a correlation had indeed occurred (Sas-Paszt et al. 2020a, b). The plants fertilized with standard fertilizers at a concentration of 60% NPK and microbiologically enriched Polifoska 6 had had a greater number of inflorescences and flowers than those that had not been fertilized in that way.

Application of beneficial microorganisms to the soil, according to Erdogan et al. (2016), causes a significant increase in strawberry fruit yields. Their positive effect on the size of the fruit and its weight, as well as on the concentrations of biologically active substances, was found by other authors (Todeschini et al. 2018; Morais et al. 2019; Kumar et al. 2019a). According to them, the effectiveness of microorganisms and their influence on individual parameters of plant growth and yielding often depend on the method of inoculation. Subsurface application mainly stimulates root growth, while foliar application intensifies the growth of aboveground parts (Şahin et al. 2019). Beneficial soil microorganisms mixed with the mineral fertilizers significantly increased the fruiting potential of strawberry plants, but to a different extent depending on the type of fertilizer and its concentration. Their reduced doses, especially of the innovative fertilizers in combination with microorganisms, had a more favorable effect on fruiting than those in the combinations where the applied fertilization was 50% higher. Because of them, the increase in the crop weight varied from 17.9% to 65%, and the mean fruit weight from 6% to 38%.depending on the type and method of fertilization, The type of fertilization had no significant effect on the physicochemical parameters of the fruit. Only the pH level of the flesh was slightly elevated under the influence of some fertilization treatments.

Many authors suggest that the implementation of natural plant cultivation technologies and plant

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fertilization with biofertilizers is an alternative to the use of high doses of mineral fertilizers, which increase plant production costs and pollute the environment, (Grzyb et al. 2012a, b; Derkowska et al. 2015; Sas-Paszt, Głuszek 2019; Sas-Paszt et al._2019a, b). Microbial bioproducts have a positive effect on the growth of crop plants, their yielding, as well as on soil microorganisms, including the arbuscular mycorrhizal fungi (Mikiciuk et al. 2019). Our own research on strawberry plants shows that the use of bioproducts and mineral fertilizers enriched with beneficial microorganisms gives specific benefits and should be more widely implemented. There is a noticeable improvement in the yielding of strawberry plants fertilized with microorganisms alone, relative to the control combination. Effective in this respect is also mineral fertilization with microbiologically enriched NPK and Urea. Under the influence of this method of fertilization, the yields produced by the strawberry plants increased by about 70%.

Güneş et al. (2009) found a beneficial effect of *Bacillus* strain FS-3 and *Aspergillus* spp. FS9 fungus on the yielding of strawberry plants above the values obtained with the use of phosphorus fertilizers alone. The authors state that the tested microorganisms can be used in the cultivation of strawberry plants to increase the availability of phosphorus to plants, increase the efficiency of using phosphorus fertilizers, reduce the costs of using phosphorus fertilizers, and to increase the yielding of strawberry plants. Similarly, other authors (Erturk et al. 2012) observed a positive effect of plant growth-promoting Rhizobacteria – *Bacillus simplex* strain RC19, *Paenibacillus polymyxa* strain RC05, and *Bacillus* sp. strain RC23 on the yielding of strawberry plants cv. 'Fern'. Inoculation with the bacteria into plant roots increased fruit yield per plant (1.98–20.85%), mean fruit weight (3.05–19.26%), and first quality fruit ratio (10.30–32.05%), compared to control. Also Rahman et al. (2018) observed a positive impact of *Bacillus amylolequefaciens* BChi1 and *Paraburkholderia fungorum* BRRh-4 on plant growth parameters, fruit yield and quality parameters in strawberry fruits. Application of these microbes increased fruit yield by up to 48%. The concentrations of phenolics, carotenoids, flavonoids and anthocyanins were significantly higher than in non-treated plants. Increased yield was observed in greenhouse production of strawberry plants inoculated *in vitro* with beneficial endophytic bacteria. Plants treated with *Arthrobacter agilis* UMCV2 and *Bacillus methylotrophicus* M4-96 produced fruit yield 42% higher than non-inoculated plants (Hernández-Soberano et al. 2020).

The issues related to the treatment of strawberry plants with the use of microorganisms that promote growth and fruiting, and improve the overall physiological condition of plants require further research.

CONCLUSION

– Soil microorganisms, including filamentous fungi and beneficial bacteria of the genus *Bacillus*, introduced into the soil without mineral fertilization, have a positive effect on improving the fruiting of strawberry plants.
– Due to the presence of microorganisms in the soil, including beneficial bacteria, fruit yield increased by 35%, and with filamentous fungi by 60%.
– A high concentration of mineral fertilizers adversely affects the biological activity of soil microorganisms.
– Beneficial bacteria and filamentous fungi added to mineral fertilizers significantly increase the fruiting potential of plants, but to a varying extent depending on the type of fertilizer and its concentration.
– Mineral fertilizers such as Urea, Polifoska 6, and Super Fos Dar 40 enriched with beneficial microorganisms and used in reduced doses of 60% promote better strawberry fruiting than when used in the full dose of 100%.
– Microorganisms, especially bacteria, increase the levels of nitrogen, potassium, magnesium, and iron in strawberry leaves.

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