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## EFFECT OF MICROBIOLOGICALLY ENRICHED FERTILIZERS ON THE YIELDING OF STRAWBERRY PLANTS UNDER FIELD CONDITIONS IN THE SECOND YEAR OF PLANTATION

### Summary

The experiment was established in the spring of 2018 in the Experimental Orchard of the Research Institute of Horticulture in Dąbrowice (Central Poland) and was carried out on strawberry plants of the cultivar 'Marmolada'. The experiment included the following experimental combinations: 1. Control – no fertilization; 2. Standard NPK fertilization (control); 3. Application of only the fungi *Aspergillus niger* and *Purpureocillium lilacinum*; 4. Application of only the bacteria *Bacillus sp.*, *Bacillus amyloliquefaciens* and *Paenibacillus polymyxa*; 5. Standard NPK + fungi; 6. Standard NPK + bacteria; 7. Polifoska 6, 100% + bacteria; 8. Urea 100% + fungi; 9. Polifoska 6, 100%; 10. Super Fos Dar 40 innovative fertilizer, 100% + bacteria; 11. Urea 60% + fungi; 12. Polifoska 6, 60% + bacteria; 13. Super Fos Dar 40 60% + bacteria. The aim of this study is to assess the impact of fertilization with innovative mineral fertilizers enriched with bacteria and filamentous fungi on the fruiting of two-year-old strawberry plants under open-field conditions. In the second year of the study, the number of inflorescences and flowers, the intensity of the green colour of the leaves, the yielding of the plants, and the quality of the fruit were determined. The measurements showed that the strawberry plants fertilized with the microbiologically enriched standard NPK fertilizer and those fertilized with a 60% dose of microbiologically enriched Polifoska 6 formed a greater number of inflorescences and flowers than the plants without fertilization. The leaves of the strawberry plants fertilized with Super Fos Dar 40 in full dose and with Polifoska 6, both microbiologically enriched, had a more intensely green colour than the control plants. The largest number of fruit was collected from the plants fertilized with the standard NPK fertilizer microbiologically enriched with the filamentous fungi. The increase in yield in this combination was as much as 123.1% in relation to the control. Comparatively high yields were also produced by the strawberry plants after the application of *Bacillus* bacteria and when fertilized with the microbiologically enriched NPK fertilizer. The largest fruits were those in the combinations where standard fertilization with the microbiologically enriched NPK fertilizer was used. The type and method of fertilizing the plants did not have a significant effect on the physicochemical properties of the fruit, such as pH, soluble-solids content, firmness and acidity.

**Key words:** fertilization, beneficial microorganisms, cropping, fruit quality, *Fragaria × ananassa*

## WPLYW NAWOZÓW WZBOGACONYCH MIKROBIOLOGICZNIE NA PLONOWANIE ROŚLIN TRUSKAWKI W WARUNKACH POŁOWYCH W DRUGIM ROKU PROWADZENIA PLANTACJI

### Streszczenie

Doświadczenie założono wiosną 2018 roku w Sadzie Doświadczalnym Instytutu Ogrodnictwa w Dąbrowicach (k. Skierniewice) i prowadzono je na roślinach truskawki odmiany 'Marmolada'. Doświadczenie objęło następujące kombinacje: 1. Kontrola – bez nawożenia; 2. Standardowe nawożenie NPK (kontrola); 3. Aplikacja grzybów strzępkowych *Aspergillus niger* i *Purpureocillium lilacinum*; 4. Aplikacja bakterii *Bacillus sp.*, *Bacillus amyloliquefaciens* i *Paenibacillus polymyxa*. 5. Standardowe nawożenie NPK + grzyby; 6. Standardowe nawożenie NPK + bakterie; 7. Polifoska 6, 100% + bakterie; 8. Mocznik 100% + grzyby; 9. Polifoska 6, 100%; 10. Super Fos Dar 40, 100% + bakterie; 11. Mocznik 60% + grzyby; 12. Polifoska 6, 60% + bakterie; 13. Super Fos Dar 40 60% + bakterie. Celem pracy była ocena wpływu innowacyjnych nawozów wzbogaconych mikrobiologicznie o bakterie i grzyby strzępkowe na owocowanie dwuletnich roślin truskawki w warunkach polowych. W drugim roku prowadzenia badań określono liczbę kwiatostanów i kwiatów, natężenie zielonej barwy liści, plonowanie roślin i jakość owoców. Pomiar wykazały, że rośliny truskawki nawożone standardowo nawozami NPK wzbogaconymi mikrobiologicznie oraz nawożone 60% dawką Polifoski 6 wzbogaconej mikrobiologicznie uformowały większą liczbę kwiatostanów i kwiatów niż rośliny bez nawożenia. Liście roślin truskawki nawożone nawozem Super Fos Dar 40 w pełnej dawce oraz Polifoską 6 wzbogaconych mikrobiologicznie miały bardziej intensywne zielone liście niż rośliny kontrolne. Najwięcej owoców zebrano z roślin nawożonych standardowo nawozami NPK wzbogaconymi mikrobiologicznie o grzyby strzępkowe. Przyrost plonu w tej kombinacji wyniósł aż 123,1% w odniesieniu do kontroli. Bardzo dobrze plonowały także truskawki po zastosowaniu bakterii z rodzaju *Bacillus* oraz nawożone standardowo nawozami NPK wzbogaconymi mikrobiologicznie. Najlepiej wyrastały owoce w kombinacjach, gdzie zastosowano standardowe nawożenie nawozami NPK wzbogaconymi mikrobiologicznie. Rodzaj i sposób nawożenia roślin nie miał istotnego wpływu na fizykochemiczne właściwości owoców, takie jak: pH, poziom ekstraktu, jędrność i kwasowość.

**Słowa kluczowe:** nawożenie, pożyteczne mikroorganizmy, plonowanie roślin, jakość owoców, *Fragaria × ananassa*

## 1. Introduction

According to global trends, farmers are becoming more and more aware of the need to reduce the use of chemicals, mainly mineral fertilizers, in agricultural production. Due to the harmfulness of the excessive use of chemicals in plant production, there is a need to replace them with organic products that are safer for the environment and human health [1, 2, 3]. One of the methods to reduce the amount of fertilizers used is to increase their effectiveness by taking advantage of beneficial microorganisms, such as filamentous and arbuscular fungi (AMF) and bacteria that are responsible for improving the growth and physiological condition of plants. Such microorganisms are also recommended to protect plants against diseases and pests, and to improve soil quality [4, 5, 6, 7, 8].

The use of mineral fertilizers in high doses additionally increases the cost of plant production and is the cause of eutrophication of surface and ground waters, degradation and pollution of the soil environment, drinking water and air [9]. An alternative to this approach is the implementation of modern plant cultivation technologies, especially fertilization, based on bio-fertilizers and soil conditioners derived from natural raw materials [4, 7, 8, 10, 11, 12, 13, 14, 15, 16]. Products of this kind have a positive effect on the growth and yielding of crop plants [17, 18, 19]. Recent scientific reports indicate that the use of fertilizers enriched with beneficial bacterial and fungal strains increases the effectiveness of such fertilizers in plant cultivation [4, 20, 21, 22]. Soil microorganisms strengthen the physiological condition of plants and also increase their resistance to biotic and abiotic environmental stresses [23, 24, 25, 26]. For example, it has been found that beneficial bacteria of the genus *Bacillus*, producing the auxin IAA and solubilizing phosphorus compounds, had a positive effect on the number, length and dry weight of roots, and also on the number of leaves and the length of petioles of strawberry plants [27].

Environmentally friendly fertilizers that will replace increasingly difficult to obtain farmyard manure and composts of plant origin are being sought for intensive agriculture, large-area farms in particular, but also for organic farms [1,11]. One of the innovative solutions is to enrich mineral and organic fertilizers and composts, as well as liquid plant growth stimulants, with consortia of beneficial microorganisms [7, 8, 28, 29, 30]. The use of native filamentous fungi and beneficial strains of bacteria in new bioproducts introduced on the market provides plants with better living conditions and promotes mineral nutrition of plants [31]. Taking advantage of such microorganisms in agriculture can lead to a significant reduction in the use of high doses of chemical products generally considered harmful to the environment [3, 7, 8, 32, 33, 34, 35].

The root system of plants, especially its morphological features, is also modified by various abiotic and biotic factors [7, 8, 36]. Numerous research studies have shown that colonization of the root system by AM fungi can change its morphological structure, including the size of the roots, their topographic arrangement, as well as their surface area and volume [7, 8, 16, 32, 37]. The presence of beneficial microorganisms in the soil also alleviates the impact of adverse environmental factors acting on the plant [3, 23].

Currently, there are not many experiments assessing the impact of the application of microbiologically enriched mineral fertilizers on the yielding of strawberry plants. Also, there are not many research results on the effectiveness

of the application to the soil of beneficial microorganisms without additional mineral fertilization in the cultivation of strawberry plants. The development of new, environmentally friendly technologies requires conducting research on the application of microbiologically enriched mineral fertilizers and the impact of such treatments on strawberry fruit yield and fruit quality. The aim of this study is to assess the impact of fertilization with innovative mineral fertilizers enriched with bacteria and filamentous fungi on the fruiting of two-year-old strawberry plants under open-field conditions.

**Research hypothesis:** Innovative microbiologically enriched fertilizers favourably affect the yielding of strawberry plants in comparison with the control treatment without fertilization and with NPK fertilization

## 2. Materials and methods

The experiment was established in the spring of 2018 in the Experimental Orchard of the Research Institute of Horticulture in Dąbrowice (Central Poland, 51° 91 41 88 N, 20° 11 13 89 E, 145 m a.s.l.) and will run for three consecutive years. 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. The soil pH was slightly acidic at pH 6.2 (in KCl), and the average humus content of the soil was 1.2%. The levels of minerals in the soil, including macro elements, was as follows: P – 7.5; K – 12.4; Mg – 5.8 mg/100 g, and microelements: B – 2.4; Cu – 4.8; Fe – 862; Mn – 75.5; Na – 4.35; Zn – 3.7 mg/1000 g.

Thermal and humidity conditions: the average temperature in the individual months of the 2019 season was: April 10°C, May 13°C, June 22°C, July 19°C, August 20°C, September 14°C, October 10°C; the average relative humidity in those months was – April 50%, May 60%, June 50%, July 60%, August 50%, September 55%, October 60%; sunshine hours – monthly totals: April 257, May 194, June 386, July 242, August 274, September 163, October 141 (<https://meteoblue.com/pl/pogoda/>).

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.

The experiment included the following experimental combinations:

1. Control (no treatment) – unfertilized podzolic soil (mineral composition given above).
2. Control – standard NPK 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. Beneficial soil fungi 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. Beneficial bacteria 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. Standard NPK fertilization as in point 2 with the beneficial soil fungi listed in point 3.
6. Standard NPK fertilization as in point 2 and the beneficial

bacteria applied to the soil as in point 4.

7. Polifoska 6 in a 100% dose, including three strains of bacteria of the genera *Bacillus* and *Paenibacillus*, which had been incorporated into the fertilizer during the formation of granules. For each plot, Polifoska 6 was used in the amount of 26 g; Urea in the amount of 30 g was applied once before planting, 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 in a 100% dose 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, and 12 g of Super Fos Dar 40 fertilizer were used. Urea in the amount of 20 g was also applied in mid-summer.

9. Polifoska 6 in a 100% dose. 26 g of Polifoska 6, 33 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 in a 100% dose enriched with three strains of *Bacillus* and *Paenibacillus* bacteria was applied in the amount of 3.83 g per plot. In addition, before planting the plants, the soil was fertilized with 50 g of potassium salt, 12 g of Super Fos Dar 40 fertilizer, and Urea – 35 g, 20 g in mid-summer.

11. Urea in a 60% dose enriched with strains of filamentous fungi of the species and quantitative composition as in point 3. For each plot before planting the plants, 30 g of potassium salt, 20 g of Urea and 7 g of Super Fos Dar 40 were used Urea in the amount of 12 g was also applied in mid-summer.

12. Polifoska 6 in a 60% dose enriched with three strains of bacteria of the genera *Bacillus* and *Paenibacillus* was used in the same way as in point 9. 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 in a 60% dose enriched with three *Bacillus* and *Paenibacillus* bacterial strains was used in the same way as in point 12. 7 g of Super Fos Dar 40, 30 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.

During the growing season, the weeds on the experimental plots were removed by hand. During periods of water deficit, the plantation was drip irrigated in accordance with the indications of tensiometers. In the first year after planting, the inflorescences were systematically removed so that the fruit would not inhibit the growth of the young plants. The influence on the growth of strawberry plants of the microbiologically enriched fertilizers and microorganisms applied to the soil on their own was assessed 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. Intensity of the green colour of leaves was also assessed.

## 2.1. Measurements and observations made

### 2.1.1. Assessment of flowering intensity

Plants from each plot were assessed for flowering intensity three times at the end of May / beginning of June at 5-day intervals until the end of fruiting, in terms of the number of inflorescences and flowers on them. The results are given as average numbers for each fertilization combination.

### 2.1.2. Assessment of fruit yield and mean fruit weight, and measurements of the green colour of leaves

Strawberry fruits from each plot were harvested four times at intervals of 4-5 days. Each time, the following parameters were determined for individual plots:

- number of fruits collected,
- weight of the fruits in grams (mean fruit weight was calculated by dividing the total fruit weight by the number of fruits),
- intensity of the green colour of the leaves was determined with a SPAD 502 meter using a sample of 5 leaves taken from two plants in each replication. The final result for each fertilization combination was based on 30 measurements.

### 2.1.3. Determination of physico-chemical parameters of strawberry fruits

**Soluble solids content** was determined using a Mettler Toledo type RE50 refractometer. The measurements were carried out in triplicate on samples obtained after grinding 10 fruits taken at random from each replication. The results were expressed in °Brix.

**Titrate acidity** was determined in accordance with the Polish standard PN-90/A-75101/04 using a Mettler Toledo type DL 58 titrator. The measurements were carried out in triplicate on samples obtained after grinding 10 fruits taken at random from each replication. 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 carried out in triplicate on the same fruits that were used to measure titrate acidity and soluble solids content.

**Fruit firmness** was expressed as the force needed to pierce the skin of the fruit with a Ø3.2 mm flat plunger moving at a speed of 50 mm/min. The measurements were carried out 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 from each replication, on the side surface corresponding to the largest diameter. The results are expressed in Newtons (N).

### 2.1.4. Statistical analysis

The results were statistically analyzed using one-way analysis of variance with the Tukey test,  $\alpha = 0.05$ , using the statistical program Statistica 13.1. Data not significantly different from each other are marked with the same letters.

### 2.1.5. Characteristics of Super Fos Dar 40 fertilizer, Polifoska 6 and Urea

**Super Fos Dar 40** belongs to the group of the most concentrated phosphate fertilizers. It contains 40% P<sub>2</sub>O<sub>5</sub> – phosphorus pentoxide soluble in mineral acids; 25% P<sub>2</sub>O<sub>5</sub> soluble in neutral citrate solution and water; 10% CaO – calcium oxide soluble in water, and microelements (Co, Cu, Fe, Mn, Zn), which are a valuable addition derived from natural phosphates that improves the assimilation of other ingredients.

**Polifoska 6** is a granulated fertilizer containing 6% nitrogen (N) in ammonium form, 20% phosphorus (P<sub>2</sub>O<sub>5</sub>), 30% potassium (K<sub>2</sub>O) in the form of a potassium salt, and 7% water-soluble sulphur trioxide (SO<sub>3</sub>) in the form of sulphate.

**Urea** is a granulated fertilizer from the group of nitrogen fertilizers, containing 46% N in amide form.

## 3. Results

The open-field experiment on strawberry plants showed that the type and method of fertilization had an impact on

the number of inflorescences and flowers produced by the plants, as well as on the chlorophyll content in their leaves (Table 1). The strawberry plants fertilized with the microbiologically enriched standard NPK fertilizer and those fertilized with a 60% dose of microbiologically enriched Polifoska 6 produced a greater number of inflorescences and flowers than the plants in the control combination (without fertilization). A similar effect was obtained by fertilizing strawberry plants with microbiologically enriched Super Fos Dar 40 fertilizer at a concentration of 100%. The results obtained in this respect, however, did not differ significantly from those for the combination where standard NPK mineral fertilization was applied. The leaves of the strawberry plants fertilized with the microbiologically enriched Super Fos Dar 40 fertilizer in full dose and microbiologically enriched Polifoska 6 had a more intense green colour than those of the control plants, which is evidence of a good supply of nitrogen to them.

The lowest-yielding strawberry plants were those in the control combination (without fertilization). The highest number of fruits were collected from the plants fertilized with the microbiologically enriched NPK fertilizer. The increase in yield in that combination was as much as 123.1% (Table 2). Very good yields were also obtained from the strawberry plants fertilized with *Bacillus* bacteria and those fertilized with the microbiologically enriched standard NPK fertilizer. In comparison with the control combination (without fertilization), the use of microbiologically enriched Polifoska 6 and Super Fos Dar 40 fertilizers in doses reduced to 60% had a negligible effect on fruit yield. The strawberry plants subjected to fertilization with microbiologically enriched Polifoska 6 at a concentration of 100%, microbiologically enriched Urea at 100%, and also Polifoska 6 at 100% or Super Fos Dar 40 at 100%, both microbio-

logically enriched, yielded better than the plants in the control combination, but not as well as those fertilized with the microbiologically enriched standard NPK fertilizer.

The fruits of unfertilized strawberry plants were the smallest. The use of Urea in a 60% dose and Super Fos Dar 40 at 60%, both microbiologically enriched, did not cause a significant increase in mean fruit weight in comparison with the control combination (without fertilization). The largest strawberry fruits developed in the combinations where standard, microbiologically enriched NPK fertilization was applied – weight gain of 32.6%, and in those with NPK mineral fertilization alone – weight gain of 26.9% (Table 2).

The method of fertilization did not affect the physico-chemical properties of the fruit. All the measurements of pH, soluble solids, titratable acidity and fruit firmness in individual combinations were within the statistical error (Table 3).

#### 4. Discussion

Worldwide surveys indicate that farmers are becoming more aware of the need to reduce the use of chemical products, including fertilizers, in agricultural production and replace them with organic products [1, 2, 3]. Therefore, efforts are being made to employ beneficial microorganisms for this purpose. Increasing the effectiveness of fertilizers in stimulating plant growth and improving their fruiting, and even replacing mineral fertilization can be achieved by using bacteria and mycorrhizal and filamentous fungi. They exert a positive effect, like soil conditioners, on the physiological condition of plants, stimulate their growth and yielding, and serve to improve soil quality, as well as protecting plants against diseases and pests [4, 5, 6, 7, 8].

Table 1. Effect of microbiologically enriched mineral fertilizers on the number of inflorescences and flowers and the chlorophyll content in the leaves of 'Marmolada' strawberry plants (IO Experimental Orchard, Dąbrowice, 2019)

Tab. 1. Wpływ nawozów mineralnych wzbogaconych mikrobiologicznie na liczbę kwiatostanów i kwiatów oraz zawartość chlorofilu w liściach roślin truskawki odmiany 'Marmolada' (Sad Doświadczalny IO, Dąbrowice, 2019 r.)

No	Treatment	Number of inflorescences per plant	Number of flowers per plant	Chlorophyll content in leaves [SPAD]
1.	Control (no fertilization)	6.8 a	35.4 a	32.4 a
2.	Control NPK	8.6 b	43.2 c	32.4 a
3.	Fungal strains	8.4 ab	38.4 ab	34.9 ab
4.	Bacterial strains	8.3 ab	44.8 cd	36.3 ab
5.	NPK + fungal strains	8.9 b	48.0 d	34.5 ab
6.	NPK + bacterial strains	8.8 b	47.8 d	35.0 ab
7.	Polifoska 6 100% + bacterial strains	7.0 a	34.4 a	36.2 b
8.	Urea 100% + fungal strains	8.0 ab	42.4 b	35.4 ab
9.	Polifoska 6 100%	7.9 ab	42.2 b	32.1 a
10.	Super Fos Dar 40 100% + bacterial strains	8.5 b	43.6 c	37.2 b
11.	Urea 60% + fungal strains	7.4 ab	47.2 cd	35.0 ab
12.	Polifoska 6 60% + bacterial strains	8.7 b	43.2 c	33.8 ab
13.	Super Fos Dar 40 60% + bacterial strains	7.6 ab	39.4 ab	31.6 a

\* Means marked with the same letters in a column do not differ significantly at  $\alpha = 0.05$ .

Source: own study / Źródło: opracowanie własne

Table 2. Effect of microbiologically enriched mineral fertilizers on fruit yield and mean fruit weight of 'Marmolada' strawberry plants (IO Experimental Orchard, Dąbrowice, 2019)

Tab. 2. Wpływ nawozów mineralnych wzbogaconych mikrobiologicznie na wielkość plonu i masę owoców roślin truskawki odmiany 'Marmolada' (Sad Doświadczalny IO, Dąbrowice, 2019 r.)

No	Treatment	Yield [g per 12 plants]	Yield [%]	Mean fruit weight [g]	Mean fruit weight [%]
1.	Control (no fertilization)	3260 a	100.0	12.81 a	100.0
2.	Control NPK (fertilizer combination)	5370 bd	164.7	16.25 de	126.9
3.	Fungal strains	6060 de	185.9	15.85 d	123.7
4.	Bacterial strains	6966 ef	213.7	15.30 cd	119.4
5.	NPK + fungal strains	7274 f	223.1	14.97 bc	116.9
6.	NPK + bacterial strains	6217 d-f	190.7	16.98 e	132.6
7.	Polifoska 6 100% + bacterial strains	4773 bc	146.4	15.46 cd	120.7
8.	Urea 100% + fungal strains	4804 bc	147.4	15.59 cd	121.7
9.	Polifoska 6 100%	4550 b	138.2	15.21 cd	118.7
10.	Super Fos Dar 40 100% + bacterial strains	4550 b	139.6	14.70 bc	114.8
11.	Urea 60% + fungal strains	5941 c-e	182.2	13.87 ab	108.3
12.	Polifoska 6 60% + bacterial strains	4375 ab	134.2	14.31 b	111.7
13.	Super Fos Dar 40 60% + bacterial strains	4398 ab	134.9	13.77 ab	107.5

\* Means marked with the same letters in a column do not differ significantly at  $\alpha = 0.05$ .

Source: own study / Źródło: opracowanie własne

Table 3. Effect of microbiologically enriched mineral fertilizers on the pH, soluble solids content, titratable acidity and firmness of 'Marmolada' strawberry fruit (IO Experimental Orchard, Dąbrowice, 2019)

Tab. 3. Wpływ nawozów mineralnych wzbogaconych mikrobiologicznie na odczyn, poziom ekstraktu, kwasowość miareczkową oraz jędrność owoców truskawki odmiany 'Marmolada' (Sad Doświadczalny IO, Dąbrowice, 2019 r.)

No	Treatment	pH	Soluble solids [°Brix]	Titratable acidity [g/100 g]	Firmness [N]
1.	Control (no fertilization)	3.69 a	9.10 a	0.76 a	1.65
2.	Control NPK (fertilizer combination)	3.78 a	9.14 a	0.79 a	1.36
3.	Fungal strains	3.82 a	8.84 a	0.72 a	1.37
4.	Bacterial strains	3.81 a	9.72 a	0.75 a	1.37
5.	NPK + fungal strains	3.83 a	9.47 a	0.74 a	1.48
6.	NPK + bacterial strains	3.86 a	10.22 a	0.72 a	1.65 a
7.	Polifoska 6 100% + bacterial strains	3.82 a	9.49 a	0.74 a	1.37
8.	Urea 100% + fungal strains	3.83 a	10.34 a	0.81 a	1.52
9.	Polifoska 6 100%	3.79 a	9.20 a	0.71 a	1.42
10.	Super Fos Dar 40 100% + bacterial strains	3.78 a	9.73 a	0.80 a	1.40
11.	Urea 60% + fungal strains	3.85 a	10.11 a	0.73 a	1.41
12.	Polifoska 6 60% + bacterial strains	3.85 a	9.89 a	0.75 a	1.32 a
13.	Super Fos Dar 40 60% + bacterial strains	3.86 a	10.32	0.74 a	1.28 a

\* Means marked with the same letters in a column do not differ significantly at  $\alpha = 0.05$ .

Source: own study / Źródło: opracowanie własne

The overall biomass of plants treated with beneficial microorganisms and the chlorophyll content are also greater, although these differences are usually not statistically significant [38, 39]. Improvements in vegetative growth parameters of strawberry plants and a higher chlorophyll content after the application of microorganisms has been observed by Karlidag et al. [40]. Better fruiting of strawberry plants after the application of microorganisms has been found by Erdogan et al. [41]. Srivastav et al. [42], in turn, have reported that in the combinations where strawberry plants were fertilized with microorganisms and with microbiologically enriched organic fertilizers, the results obtained were a higher number of flowers per plant, earlier flowering, increased number of fruits, and a higher percentage of fruit setting per plant. In addition, the harvesting period was longer than for the control combination. The results of our own research indicate a positive effect of microbiologically enriched mineral fertilizers in the open-field cultivation of

strawberry. The plants fertilized with the microbiologically enriched standard NPK fertilizer and those fertilized with a 60% dose of microbiologically enriched Polifoska 6 produced a greater number of inflorescences and flowers than the plants without fertilization. The leaves of the strawberry plants fertilized with the fertilizer Super Fos Dar 40 in full dose and with Polifoska 6, both microbiologically enriched, had leaves of a more intense green colour than the control plants, which indicates a good supply of nitrogen to them.

Positive effects of microorganisms on the size of fruits and their weight, as well as on the amounts of biologically active substances in them have been found by Kumar et al. [43] and Morais et al. [44]. The effectiveness of microorganisms and their influence on individual growth and yield parameters often depend on the inoculation method. Application to the soil mainly stimulates root growth, whereas foliar application increases the growth of the aerial parts [45]. In our experiment, the lowest-yielding plants were

those without fertilization, whereas the highest increase in yield was obtained from the plants fertilized with NPK in combination with filamentous fungi. Comparatively high fruit yields were also obtained from the plants fertilized with *Bacillus* bacteria and with the microbiologically enriched standard NPK fertilizer. The fruits of the strawberry plants grown without fertilization were the smallest, while the best-developed were those from the combinations where standard NPK fertilization together with bacteria was used. No significant influence of the experimental combinations on the physico-chemical properties of strawberry fruit was found.

Many authors are of the opinion that the implementation of natural plant cultivation technologies and fertilization of crop plants with bio-fertilizers represent the best alternative to the use of high doses of mineral fertilizers that increase plant production costs and pollute the environment is [4, 7, 8, 10, 11, 12, 13, 14, 15, 16, 22, 46]. Bioproducts of this type have a positive effect on the growth of crop plants, their yielding, as well as on soil microorganisms and fauna, including the development of arbuscular mycorrhizal fungi [17, 18, 19, 47]. Our research on strawberry plants shows that the use of bioproducts and microbiologically enriched mineral fertilizers brings benefits and should be more widely propagated. It is particularly noteworthy that the yielding of strawberry plants fertilized with microorganisms is improved in relation to the control combination. NPK mineral fertilization and with Urea, both microbiologically enriched, were also effective in this respect. Under the influence of this method of fertilization, strawberry fruit yields were about twice as high as those in the control combination.

Güneş et al. [48] have found a beneficial effect of the bacterium *Bacillus* FS-3 and the fungus *Aspergillus* FS9 on strawberry fruit yield above the values obtained after using phosphorus fertilizers only. The authors pointed out that the tested microorganisms could be employed in the cultivation of strawberry plants to increase the availability of phosphorus to them, increase the efficiency of using phosphorus fertilizers and reduce the cost of using them, and to increase the yielding of strawberry plants. Similarly, Erturk et al. [49] have observed a positive effect of plant growth-promoting Rhizobacteria – *Bacillus simplex* RC19, *Paenibacillus plymyxa* RC05, and *Bacillus* sp. RC23 on the yielding of strawberry plants of the cultivar ‘Fern’. Inoculation of the bacteria into the roots of the plants increased the fruit yield per plant (1.98–20.85%), mean fruit weight (3.05–19.26%), and first quality fruit ratio (10.30–32.05%) in relation to the control.

Generally, the results of our study on the impact of the use of microorganisms and mineral fertilizers enriched with them on the yielding of strawberry plants under open-field conditions are consistent with the observations of the above-cited authors mainly in the part that concerns fertilization with microbiologically enriched fertilizers. In the available literature, there are no reports on in-depth research on the effects of introducing microorganisms on their own directly into the soil on the fruiting of strawberry plants without additional mineral fertilization. The topics related to fertilizing strawberry plants with the use of microorganisms promoting growth and fruiting as well as improving the overall physiological condition of plants require further research.

## 5. Conclusions

Fertilizing strawberry plants with the bacteria and filamentous fungi, and also with the microbiologically enriched NPK fertilizer had a beneficial effect on fruit yield. In these combinations, the yield increased by over 100 percent. Fertilization with the fertilizers Polifoska 6 and Super Fos Dar 40, both microbiologically enriched, also increased the yielding of strawberry plants, but to a much lesser extent than fertilization with the bacteria and filamentous fungi only, and the microbiologically enriched NPK fertilizer. The fertilization combinations used were found to have an effect on fruit quality. Strawberry fruit grew best on the plots where microbiologically enriched standard NPK fertilization was applied. The physico-chemical properties of the fruit did not change significantly under the influence of the experimental combinations.

## 6. References

- [1] Arancon N.Q., Edwards C.A., Berman P., Welch C., Metzger J.D.: Influence of vermicomposts on field strawberries: 1. Effects on growth and yields Bioresource Technology, 2004, 93: 145-153.
- [2] Chang E.H., Chung R.S, Tsai Y.H.: Effect of different application rates of organic fertilizer on soil enzyme activity and microbial population. Soil Sci. Plant Nutr., 2007, 53: 132-140.
- [3] Sas-Paszt L., Sumorok B., Malusa E., Głuszek S., Derkowska E.: The influence of bioproducts on root growth and mycorrhizal occurrence in the rhizosphere of strawberry plants ‘El-santa’. J. Fruit Orn. Plant Res., 2011, 19(1): 13-33.
- [4] Gousterova A., Nustorova M., Christov P., Nedkov P., Neshev G., Vasileva-Tonkova E.: Development of biotechnological procedure for treatment of animal wastes to obtain inexpensive biofertilizer. World J. Microbiol. Biotechnol., 2008, 24: 2647-2652.
- [5] Meszka B., Bielenin A.: Bioproducts in control of strawberry verticillium wilt. Phytopathologia, 2009, 52: 21-27.
- [6] Dobrzyński J., Jankiewicz U., Sitarek M., Stępień W., Sas-Paszt L., Górska E.B.: Występowanie względnie beztlenowych, przetrwalnikujących bakterii celulolitycznych w glebie nawożonej kompostami przygotowanymi z miału węgla brunatnego. Konferencja Naukowa „Ocena gleb użytkowanych rolniczo” IUNG-PIB 26-27.06. 2014, Puławy, 90.
- [7] Sas-Paszt L., Sumorok B., Derkowska E., Trzciniński P., Lisek A., Grzyb S. Z., Sitarek M., Przybył M., Frąc M. Effect of microbiologically enriched fertilizers on the vegetative growth of strawberry plants under field conditions in the first year of plantation. J. Res. Applic. Agric. Engng, 2019 a, Vol. 64 (2): 29-37.
- [8] Sas-Paszt L., Sumorok B., Derkowska E., Trzciniński P., Lisek A., Grzyb S. Z., Sitarek M., Przybył M., Frąc M. Effect of microbiologically enriched fertilizers on the vegetative growth of strawberry plants in container-based cultivation at different levels of irrigation. J. Res. Applic. Agric. Engng, 2019 b, Vol. 64 (2): 38-46.
- [9] Boy J., Arcand Y.: Current trends in green technologies in food production and processing. Food Eng. Rev., 2013, 5: 1-17.
- [10] Chelariu E.L., Ionel A.: Results regarding the influence of fertilization with Vinassa Rompak upon the crop yield at Sante potato species. 4th International Symposium, Buletinul U.S.A.M.V Cluj-Napoca, 2005, vol. 61, 408, Seria Agricultura.
- [11] Malusa E., Sas-Paszt L., Popińska W., Żurawicz E.: The effect of a substrate containing arbuscular mycorrhizal fungi and rhizosphere microorganisms (*Trichoderma*, *Bacillus*, *Pseudomonas* and *Streptomonas*) and foliar fertilization on growth response and rhizosphere pH of the tree strawberry cultivars. Inter. J. Fruit Sci., 2007, 6: 25-41.

- [12] Sas-Paszt L., Żurawicz E., Filipeczak J., Gluszek S.: Rola ryzofery w odżywianiu roślin truskawki. *Post. Nauk Rol.*, 2008, 6: 27-36.
- [13] Khan W., Rayirath U.P., Subramanian S., Jithesh M.N., Rayorath P., Hodges D.M., Critchley A.T., Craigie J.S., Norie J., Prithiviraj B.: Seaweed Extracts as biostimulants of plant growth and development. *J. Plant Growth Regul.*, 2009, 28: 386-399.
- [14] Malusa E., Sas-Paszt L.: The development of innovative technologies and products for organic fruit production. An Inte-grated Project. The Proceedings of the International Plant Nutrition Colloquium XVI, 2009, Paper: 1359, 1-3. <http://schol-arship.org/uc/item/5f10g7pg>.
- [15] Grzyb Z.S., Bielicki P., Piotrowski W., Sas-Paszt L., Malusa E.: Effect of some organic fertilizers and amendments on the quality of maidens trees of two apple cultivars. *Proc. 15th Intern. Confer. on Organic Fruit Growing*, 20th-22th February 2012; (Univ. of Hohenheim, Germany), 2012a, 410-414.
- [16] Derkowska E., Sas-Paszt L., Trzciński P., Przybył M., Weszczak K.: Influence of biofertilizers on plant growth and rhizosphere microbiology of greenhouse-grown strawberry cultivars. *Acta Sci. Pol. Hortorum Cultus*, 2015a, 14(6): 83-96.
- [17] Kuwada K., Kuramoto, M., Utamura M., Matsusita I., Shibata Y., Ishii T.: Effect of mannitol from *Laminaria japonica*, other sugar alcohols, and marine alga polysaccharides on *in vitro* hyphal growth of *Gigaspora margarita* and root colonization of trifoliolate orange. *Plant Soil*, 2005, 276: 279-286. <http://dx.doi.org/10.1007/s11104-005-4985-2>.
- [18] Kuwada K., Wamocho L.S., Utamura M., Matsushita I., Ishii T. Effect of red and green algal extract on hyphal growth of arbuscular mycorrhizal fungi and on mycorrhizal development and growth of papaya and passion fruit. *Agronom. J.*, 2006, 98: 1340-1344. [doi.org/10.2134/agronj2005.0354](http://dx.doi.org/10.2134/agronj2005.0354).
- [19] [19]Smith S.E., Read D.J., *Mycorrhizal Symbiosis*. 3rd Edition Elsevier and Academic, New York, London, Burlington, San Diego, 2008.
- [20] Chen J.: The combined use of chemical and organic fertilizers and/ or fertilizer for crop growth and soil fertility . International Workshop on Sustained Management of the Soil-Rizosphere System for Efficient Crop Production and Fertilizer Use, Bangkok, 2006, 1-11.
- [21] Chelariu E.L., Draghia L., Bireescu G., Bireescu L., Branza M.: Research regarding the influence of Vinassa fertilization on *Gomphrena globosa* species. *Lucr. ętiințifice*, Ed. Ion Ionescu de la Brad, Iași Usamv Iasi, Seria Horticultura, 2009, 52: 615-620.
- [22] Derkowska E., Sas-Paszt L., Dyki B., Sumorok B.: Assessment of mycorrhizal frequency in the roots of fruit plants using different dyes. *Adv. Microbiol.*, 2015b, 5(1): 54-64.
- [23] Stewart L., Hamel C., Hogue R., Moutoglis P.: Response strawberry mycorrhizal fungi under very high soil phosphorus conditions. *Mycorrhiza*, 2005, 15: 612-619.
- [24] Yin B., Wang Y., Liu P., Hu J., Zhen W.: Effects of vesicular-arbuscular mycorrhiza on the protective system in strawberry leaves under drought stress. *Front. Agric. China*, 2010, 4: 165-169.
- [25] Corte L., Dell'Abate M.T., Magini A., Migliore M., Felici B., Roscini L., Sardella R., Tancini B., Emiliani C., Cardinali G., Benedetti A.: Assessment of safety and efficiency of nitrogen organic fertilizers from animal-based protein hydrolysates – A Laboratory Multidisciplinary Approach, 2013. *J. Sci. Food Agric.*, 2013, 94: 235-245. <http://dx.doi.org/10.1002/jsfa.6239>.
- [26] Wally O.D., Critchley A., Hiltz D., Craigie J., Han X., Zaharia L.I., Abrams S., Prithiviraj B.: Regulation of phytohormone biosynthesis and accumulation in *Arabidopsis* following treatment with commercial extract from the marine macroalga *Ascophyllum nodosum*. *J. Plant Growth Regul.*, 2013, 32: 324-339. [doi.org/10.1007/s00344-012-9301-9](http://dx.doi.org/10.1007/s00344-012-9301-9).
- [27] Dias A.C., Costa F.E., Andreote F.D., Lacava P.T., Teixeira M.A., Assumpção L.C., Araujo W.L., Azevedo J.L., Melo I.S.: Isolation of micropropagated strawberry endophytic bacteria and assessment of their potential for plant growth promotion. *World Journal of Microbiology and Biotechnology*, 2009, 25(2): 189-195.
- [28] Hodge A., Campbell C.D., Fitter A.H.: An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature*, 2001, 413: 297-299.
- [29] Ravnkov S., Jensen B., Knudsen I.M., Bodker L., Funck Jensen D., Karlinski L., Larsen J.: Soil inoculation with the biocontrol agent *Clonostachys rosea* and the mycorrhizal fungus *Glomus intraradices* results in mutual inhibition, plant growth promotion and alteration of soil microbial communities. *Soil. Biol. Biochem.*, 2006, 38: 3453-3462.
- [30] Sas-Paszt L., Malusa E., Sumorok B., Canfora L., Derkowska E., Gluszek S.: The influence of bioproducts on mycorrhizal occurrence and diversity in the rhizosphere of strawberry plants under controlled conditions. *Adv. Microbiol.*, 2015, 5 (1): 40-53.
- [31] Regvar M., Vogel-Mikuš K., Ševerkar T. Effect of AMF inoculums from field isolates on the yield of green pepper, parsley, carrot and tomato. *Folia Geobot.*, 2003, 38, 223-234.
- [32] Derkowska E., Sas-Paszt L., Sumorok B., Dyki B.: Colonization of apple and blackcurrant roots by arbuscular mycorrhizal fungi following mycorrhization and the use of organic mulches. *Folia Hort.*, 2013, 25(2): 117-122.
- [33] Lingua G., Bona E., Manassero P., Marsano F., Todeschini V., Cantamessa S., Copetta A., D'Agostino G., Gamalero E., Berta G.: Arbuscular mycorrhizal fungi and plant growth-promoting pseudomonads increases anthocyanin concentration in strawberry fruits (*Fragaria x ananassa* var. Selva) in conditions of reduced fertilization. *Int. J. Mol. Sci.*, 2013, 14: 16207-16225. [doi:10.3390/ijms140816207](http://dx.doi.org/10.3390/ijms140816207).
- [34] Grzyb Z.S., Piotrowski W., Sas-Paszt L.: Effect of fertilization in organic nursery for later growth and fruiting of apple trees in the orchard. *J. Life Sciences*, 2015a, 9: 159-165.
- [35] Grzyb Z.S., Sas-Paszt L., Piotrowski W., Malusa E.: The influence of mycorrhizal fungi on the growth of apple and sour cherry maidens fertilized with different bioproducts in the organic nursery. *J. Life Sciences*, 2015b, 9: 221-228.
- [36] Fan L., Dalpé Y., Fang Ch., Dubé C., Kanizadeh S.: Influence of arbuscular mycorrhizae on biomass and root morphology of selected strawberry cultivars under salt stress. *Botany*, 2011, 89(6): 397-403. [doi 10.1139/b11-028](http://dx.doi.org/10.1139/b11-028).
- [37] Kapoor R., Sharma D., Bhatnagar A.K.: Arbuscular mycorrhizae in micropropagation systems and their potential applications. *Sci. Hortic.*, 2008, 116 (3): 227-239.
- [38] Singh A., Singh J.N.: Studies on influence of biofertilizers and bioregulators on flowering, yield and fruit quality of strawberry cv. Sweet Charlie. *Annals of Agricultural Research*, 2006, 27: 261-264.
- [39] Singh A., Singh J.N.: Effect of biofertilizers and bioregulators on growth, yield and nutrient status of strawberry cv. Sweet Charlie. *Indian Journal of Horticulture*, 2009, 66: 220-224.
- [40] Karlidag H., Yildirim E., Turan M., Pehlivan M., Donmez F.: Plant growth-promoting rhizobacteria mitigate deleterious effects of salt stress on strawberry plants (*Fragaria x ananassa*). *HortScience*, 2013, 48: 563-567.
- [41] Erdogan Ü., Ramazan Çakmakçı., Atafeh Varmazyari, Metin Turan, Yaşar Erdogan, Nurgül Kitir. Role of inoculation with multi-trait rhizobacteria on strawberries under water deficit stress. *Zemdirbyste-Agriculture*, 2016, 103: 67-76.
- [42] Srivastava A., Singh R., Baksh H., Singh B.K., Raj P., Pal V.K., Maurya L.K.: Effect of organic manures and bio-fertilizers on floral and commercial yield characters of strawberry (*Fragaria x ananassa* Duch.) cv. Chandler. *Inter. J. Cur. Microbiol. Appl. Sci.*, 2019, 8: 393-397.
- [43] Kumar S., Kundu M., Das A., Rakshit R., Siddiqui MdW., Rani R.: Substitution of mineral fertilizers with biofertilizer: an alternate to improve the growth, yield and functional biochemical properties of strawberry (*Fragaria x ananassa* Duch.) cv. Camarosa. *J. Plant Nutr*, 2019: 1-20.
- [44] Morais MC, Mucha Â, Ferreira H, Gonçalves B, Bacelar E, Marques G.. Comparative study of plant growth-promoting

bacteria on the physiology, growth and fruit quality of strawberry. *J. Sci. Food Agric.*, 2019, 99: 5341–5349.

- [45] Şahin M., Eşitken A., Pirlak L., Altıntaş S., Turan M.: Effect of DN1 bacterial strain applied by different methods on some morphological characteristics of strawberry cv. San Andreas (*Fragaria x ananassa* Duch.). *AGROFOR International Journal*, 2019, 4: 57-64.
- [46] Grzyb Z.S., Piotrowski W., Bielicki P., Sas-Paszt L., Malusa E.: Effect of different fertilizers and amendments on the growth of apple and sour cherry rootstock in an organic nursery. *J. Fruit Ornament. Plant Res.*, 2012b, 20(1): 43-53.
- [47] Mikiciuk G., Sas-Paszt L., Mikiciuk M., Derkowska E., Trzciniński P., Głuszek S., Lisek A., Wera-Bryl S., Rudnicka J.: Mycorrhizal frequency, physiological parameters, and yield of strawberry plants inoculated with endomycorrhizal fungi and rhizosphere bacteria. *Mycorrhiza*, 2019, 29: 489-501.
- [48] Güneş A., Ataoğlu N., Turan M., Eşitken A., Ketterings Q.M.: Effects of phosphate-solubilizing microorganisms on strawberry yield and nutrient concentrations. *Journal of Plant Nutrition and Soil Science*, 2009, 172(3): 385-392.
- [49] Erturk Y., Ercisli S., Cakmakci R.: Yield and growth response of strawberry to plant growth-promoting rhizobacteria inoculation. *J. Plant Nutr.*, 2012, 35(6): 817-826.

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