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INFLUENCE OF BIOFERTILIZERS ON PLANT GROWTH AND RHIZOSPHERE MICROBIOLOGY OF GREENHOUSE-GROWN STRAWBERRY CULTIVARS

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Abstract. The aim of this study was to assess the growth and development of plants of three strawberry cultivars fertilized with selected biofertilizers under greenhouse conditions. The experiments were conducted in a greenhouse complex of the Research Institute of Horticulture in Skierniewice from February to July in 2013 and 2014. Plants of three strawberry cultivars, 'Elsanta', 'Honeoye' and 'Elkat', were planted in rhizoboxes and grown under the following fertilization regimes: 0-control (no fertilization), NPK control, Micosat F (bacterial-mycorrhizal substrate), manure, Humus UP, and Vinassa. Applications of Humus UP resulted in beneficial effects on plant height, leaf surface area, leaf fresh and dry weight, the degree of mycorrhizal colonization in the roots, and on the number of spores of arbuscular mycorrhizal fungi in the rhizosphere of strawberry plants. Biopreparations Humus UP and Vinassa also had a positive influence on the size of the root system, the total number of bacteria, including spore-forming bacteria, and the total number of filamentous fungi in the rhizosphere soil, compared with mineral NPK fertilization under greenhouse conditions.

Key words: bioproducts, plant growth, AM fungi, rhizosphere bacteria, *Fragaria* × *ananassa*

INTRODUCTION

The fact that crop producers are well aware of the need to reduce the use of chemical means of production is conducive to the development of biological alternatives, making use of e.g. beneficial microorganisms, in the mineral nutrition of plants and stimulation of their growth and development, in plant protection, as well as in technologies for improving the quality of the soil. The need to protect the natural environment is associated with advancing degradation of soils, the negative impact of intensive crop production,

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and the worsening effects of climate change [Dobrzyński et al. 2014]. High levels of fertilization, often occurring in intensive agriculture, have a negative effect on root growth and root colonization by mycorrhizal fungi [Smith and Read 2008], and constitute not only a significant cost to crop producers but are also a potential cause of eutrophication and pollution of the soil environment, water and air [Boy and Arcad 2013]. One of the proposed solutions to environmental and human health protection issues is implementation of natural technologies of plant cultivation and fertilization through applications of biofertilizers. Products of this kind have a positive influence on the growth and yielding of crop plants as well as on the soil fauna, including the development of arbuscular mycorrhizal fungi (AMF) [Kuwada et al. 2005, 2006]. Enriching fertilizers with beneficial strains of bacteria and fungi can increase their effectiveness in crop production [Chen 2006] by enhancing the physiology of crop plants, stimulating their growth and yielding, as well as by increasing their resistance to environmental and biotic stresses [Corte et al. 2013, Wally et al. 2013]. The search for fertilizers for modern, environmentally-friendly agriculture, including organic farming, has been necessitated by the limited availability of traditional means of agricultural production, such as farmyard manure and composts of plant origin. One innovative solution involves microbiological enrichment of organic fertilizers, composts, and liquid plant growth promoters with consortia of beneficial microorganisms [Sas Paszt et al. 2015]. Application of native mycorrhizal fungi and beneficial strains of bacteria and fungi incorporated in new bioproducts ensures their better adaptation and survival in the prevailing environmental conditions, which is an extremely important factor for their long-term effects on plants [Regvar et al. 2003]. Both arbuscular mycorrhizal (AM) fungi and beneficial plant growth-promoting rhizobacteria (PGPR) can improve mineral nutrition of plants, and making use of them in agriculture can lead to a reduction in the use of chemicals in crop production [Lingua et al. 2013]. The structure of the root system, and especially its morphological characteristics, is modified by various abiotic and biotic factors [Fan et al. 2011]. Colonization of the root system by AM fungi can change its morphological structure, e.g. the size of the roots, their topographical arrangement, and also their surface area and volume [Kapoor et al. 2008], which helps in mitigating the effects of adverse environmental factors. There have been few studies on the influence of biofertilization, i.e. the use of biofertilizers enriched with beneficial soil microorganisms, on the size of the root system and the growth and yielding of crop plants. The aim of this study was to assess the growth and development of plants of three strawberry cultivars fertilized with selected biofertilizers under greenhouse conditions.

MATERIALS AND METHODS

The experiments were conducted in a glasshouse complex of the Research Institute of Horticulture in Skierniewice from February to July in 2013 and 2014. Plants of three strawberry cultivars: ‘Elsanta’, ‘Honeoye’ and ‘Elkat’, were planted in rhizoboxes filled with a podsolic soil at 2 kg per rhizobox. The soil (pH 5.5) had been collected from the Institute’s orchard and its mineral composition is presented in the table 1.

Table 1. The mineral composition of the soil used for the greenhouse experiment with strawberry plants

Organic matter %	N % ADW	C % DW	P	K	MG	CA	B	CU	FE	MN	ZN
			mg·100 g^{-1}			mg·kg^{-1}	mg·1000 g^{-1}				
1.5	0.07	0.88	15	11	6	1145	4.9	6.5	701	127	6.9

The plants were grown under a photoperiod of 16/8 h (day/night) and light intensity of 70 µmol·m^{-2}·s^{-1}, at 25/20°C and a relative humidity of 50%. The experiment included the following experimental combinations:

1. Control (no treatment) – unfertilized podsolic soil (composition given above).

2. Standard NPK soil fertilization (per plant): 4 g NH_4NO_3 · kg^{-1}, 3 g triple superphosphate · kg^{-1}, and 6 g K_2SO4 · kg^{-1}.

3. Manure – dry granulated bovine manure, suitable for organic farming (Doktor O'grodnik), containing: 55% C, 1% N, 0.3% P and 1% K; the product also contains microelements and soil microorganisms (110^6 CFU·g^{-1}). It was applied to the soil, near the root system (1 g per plant), at planting.

4. Micosat F (CCS Aosta s.r.l.) – a mixture of beneficial soil fungi and bacteria containing: five species of AM fungi: *Funneliformis mosseae* Taxtersensu Gerd. & Trappe, *Rhizophagus intraradices* Schenk & Smith, *Claroideoglomus claroideum* (Nicolson et Gerdemann) Trappe et Gerdemann, *Septoglomus viscosum* Nicolson and *Funneliformis coronatum* (Giovannetti); *Trichoderma viride* Pers.; three rhizosphere bacteria species (*Bacillus subtilis*, *Pseudomonas fluorescens*, *Streptomyces* spp.) with a total concentration of 10^6 CFU·g^{-1} of substrate. The product contains 40% C, 0.15% N, 431 mg·kg^{-1} P, and 9558 mg·kg^{-1} K. It was applied to the soil, near the root system (10 g per plant), at planting.

5. Humus UP (Ekodarpol) – an extract from a vermicompost containing 0.65% C, 0.03% N, 30.8 mg·kg^{-1} P and 4535 mg·kg^{-1} K. The product was first applied to the soil at planting as a 2% solution (15 ml per plant), and then three times during the growing period (1% solution, 15 ml per plant), at 10 days intervals.

6. Vinassa – molasses residue from yeast production containing 12.0% C, 1.86% N, 949 mg·kg^{-1} P, 17615 mg·kg^{-1} K. The product was first applied to the soil at planting as a 0.5% solution (50 ml per plant), and then three times during the growing period as a 0.2% solution (50 ml per plant), at 7 days intervals.

Each product was applied to 6 plants grown in 3 rhizoboxes (2 plants per rhizobox) arranged in a completely randomized design.

After the completion of the experiments in July 2013 and July 2014, samples of plant material and soil were collected to determine the growth characteristics of the aboveground parts and the roots of the plants, to conduct microbiological analyses of the growth substrates, and to assess the degree of mycorrhizal colonization in the roots and the number of spores of AM fungi.

Analysis of growth characteristics of aboveground parts of plants

Analysis of the leaves was performed with an EPSON EXPRESSION 10000 XL root scanner. Leaves from each strawberry plant were laid out on a tray and then scanned; their surface area was determined with WinRhizo software [Arsenault et al. 1995]. Measurements

of plant height and fresh and dry weight of leaves were performed by standard methods using a ruler and a laboratory balance (RADWAG WLC 3/A2/C/2) (tab. 2).

Determination of root growth characteristics

Strawberry roots with adhering soil were collected in July 2013 and 2014 to determine their morphological features. Each root system was placed in a sieve and gently rinsed with tap water. After drying, the roots were scanned with the EPSON EXPRESSION 10000 XL root scanner. Root growth characteristics (root length, root surface area, root diameter, root volume, and the number of root tips) were determined with WinRhizo software [Arsenault et al. 1995] (tabs 3, 4).

Microbiological analysis of growth substrates

Soil samples (5 g each), collected in July 2013 and 2014, were placed in distilled water (45 ml) so that 1 ml of suspension contained 0.1 g of soil, and then shaken for 40 minutes on a laboratory shaker (150 rpm). The resulting suspensions were used to prepare serial decimal dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}), which were dispensed onto plates containing appropriate culture media.

Total number of bacteria was estimated by plating 100 µl aliquots of suspension onto plates containing 20% Tryptone Soy Agar (TSA) [Vieira and Nahas 2005]. To estimate the total number of spore-forming bacteria, the test suspension was incubated at 80°C for 30 minutes and then dispensed in 100 µl aliquots onto plates containing 20% TSA. To estimate the total number of filamentous fungi, 100 µl aliquots of suspension were dispensed onto plates containing Rose Bengal Chloramphenicol Agar [Chang et al. 2007].

Plates with bacterial colonies were incubated for 7 days at 28°C, and the plates with fungal colonies for 5–7 days at 25°C. When calculating the number of bacteria and fungi, plates containing 30–300 and 10–50 colonies, respectively, were taken into account. The results were converted to colony forming units per 1 gram of dry weight of substrate (cfu·g^{-1} DW) (tab. 5).

Assessment of root colonization by arbuscular mycorrhizal fungi

Strawberry roots (10 g from each replication), collected in July 2013 and 2014, were stained in accordance with the method developed in the Rhizosphere Laboratory of the Institute [Derkowska et al. 2013, 2015]. Root fragments were prepared for further analyses in the following stages:

1. Maceration and clearing of root tissues with 10% sodium hydroxide (NaOH) at 65°C for up to 30 min.
2. Washing out roots from NaOH solution with water – 5 min.
3. Acidification of roots with 10% lactic acid – 10 min.
4. Staining with carbol fuchsin for up to 10 min.
5. Washing with water to remove excess dye – 10 min.
6. Preservation and storage of roots in glycerol.

Next, microscopic specimens were prepared by selecting each time 30 root fragments, approx. 1 cm long, laying them out in parallel on a glass slide containing glycerin, and crushing them with a coverslip. These histological specimens were examined with a Nikon 50i microscope (20×, 40×, 60×, 100× objectives), and the mycorrhizal structures observed were photographed. Determination of the degree of colonization of the roots by arbuscular mycorrhizal fungi was performed by the method of Trouvelot et al. [1986]. The results were used to calculate mycorrhizal frequency (F%) with a computer program MYCOCALC, available from the website: http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/MYCOCALC.EXE (tab. 6).

Table 2. Effect of biopreparations on shoot growth characteristics of Elsanta, Honeoye and Elkat strawberry plants. Analysis of the results, July 2013–2014

Treatment	Shoot height (cm)			Leaf surface area ($cm^2 \cdot plant^{-1}$)			Leaf fresh weight ($g \cdot plant^{-1}$)			Leaf dry weight ($g \cdot plant^{-1}$)		
	Elsanta	Honeoye	Elkat	Elsanta	Honeoye	Elkat	Elsanta	Honeoye	Elkat	Elsanta	Honeoye	Elkat
Control	20 a-c	18 ab	19 a-c	977 a-d	986 a-d	896 a-d	15 a-d	12 a-c	14 a-d	2.9 b-d	2.6 b-d	2.9 b-d
Control NPK	17 ab	15 a	17 ab	682 a	708 ab	778 a-c	11 a-c	8 a	11 ab	1.6 ab	1.2 a	1.9 a-c
Micosat F	24 bc	23 bc	23 bc	1279 a-d	1159 a-d	1146 a-d	17 b-d	17 b-d	16 b-d	3.5 d	2.6 b-d	2.8 b-d
Humus UP	26 c	26 c	26 c	1484 d	1112 a-d	1360 cd	19 d	15 b-d	18 cd	3.7 d	3.1 b-d	3.4 cd
Manure	21 a-c	21 a-c	21 a-c	1410 d	1226 a-d	1323 b-d	16 b-d	18 b-d	19 d	3.3 cd	3.2 cd	3.3 cd
Vinassa	23 bc	21 a-c	22 a-c	1227 a-d	1211 a-d	1135 a-d	17 b-d	19 d	14 a-d	3.3 cd	3.3 cd	2.6 b-d

Means in columns marked with the same letter do not differ significantly at p = 0.05 according to Tukey's multiple test

Table 3. Effect of biopreparations on root length, surface area and diameter of Elsanta, Honeoye and Elkat strawberry plants. Analysis of the results, July 2013–2014

Treatment	Root length ($cm \cdot plant^{-1}$)			Root surface area ($cm^2 \cdot plant^{-1}$)			Root diameter ($mm \cdot plant^{-1}$)		
	Elsanta	Honeoye	Elkat	Elsanta	Honeoye	Elkat	Elsanta	Honeoye	Elkat
Control	1767 bc	2316 c-e	2230 c-e	294 a-d	335 b-e	278 a-c	0.46 b-d	0.43 ab	0.42 ab
Control NPK	833 a	1234 ab	1792 bc	184 a	217 ab	231 ab	0.43 ab	0.38 a	0.41 ab
Micosat F	2035 cd	3213 fg	3338 fg	360 c-e	450 e-g	440 e-g	0.58 f	0.50 ce	0.51 ce
Humus UP	3608 g	3568 g	3594 g	550 g	492 fg	501 fg	0.79 g	0.79 g	0.49 c-e
Manure	2154 c-e	3174 fg	2894 e-g	411 d-f	521 fg	440 e-g	0.54 ef	0.52 ce	0.49 c-e
Vinassa	2698 d-f	3360 fg	3261 fg	430 e-g	453 e-g	413 d-f	0.58 f	0.46 b-d	0.49 c-e

Means in columns marked with the same letter do not differ significantly at p = 0.05 according to Tukey's multiple test

Table 4. Effect of biopreparations on root volume and number of root tips of Elsanta, Honeoye and Elkat strawberry plants. Analysis of the results, July 2013–2014

Treatment	Root volume ($cm^3 \cdot plant^{-1}$)			Number of root tips (per plant)		
	Elsanta	Honeoye	Elkat	Elsanta	Honeoye	Elkat
Control	3.70 a-c	3.68 a-c	3.72 a-d	2006 a-c	3102 a-d	2908 a-d
Control NPK	2.20 a	3.03 a	3.32 ab	1501 a	1634 ab	2496 a-d
Micosat F	5.80 de	5.78 de	5.18 b-e	3480 a-d	4421 c-f	4347 c-f
Humus UP	5.63 de	5.60 c-e	5.67 de	4101 b-e	6905 gh	4672 d-g
Manure	5.86 de	5.30 c-e	5.02 b-e	3141 a-d	3725 a-c	6011 e-g
Vinassa	6.41 e	5.99 e	6.18 e	6602 f-h	8538 h	4050 b-e

Means in columns marked with the same letter do not differ significantly at p = 0.05 according to Tukey's multiple test

Table 5. Effect of biopreparations on the total number of bacteria, total number of fungi, and total number of spore-forming bacteria in the soil collected from under Elsanta, Honeoye and Elkat strawberry plants. Analysis of the results in July 2013–2014

Treatment	Total number of fungi $\times 10^5$ cfu $\cdot g^{-1}$			Total number of bacteria $\times 10^6$ cfu $\cdot g^{-1}$			Total number of spore-forming bacteria $\times 10^6$ cfu $\cdot g^{-1}$		
	Elsanta	Honeoye	Elkat	Elsanta	Honeoye	Elkat	Elsanta	Honeoye	Elkat
Control	27.6 bc	31.6 de	29.4 cd	178.2 de	111.1 ab	125.5 a-c	10.2 b	9.34 b	16.1 e
Control NPK	26.8 bc	21.8 a	35.1 ef	89.9 a	244.8 f-h	184.6 d-f	14.9 de	12.6 c	17.9 f
Micosat F	27.8 b-d	45.6 h	63.6 i	111.2 ab	199 d-g	233.2 e-h	9.7 b	10.3 b	20.9 g
Humus UP	28 b-d	30.2 cd	37.8 fg	88.2 a	153.9 b-d	223.8 e-h	25.8 h	18.1 f	10.8 b
Manure	25.2 ab	34.1 e	39.2 g	83.5 a	255.1 h	394.1 i	9.7 b	20.7 g	14.7 de
Vinassa	28 b-d	34.3 ef	29.9 cd	165.1 cd	241.3 h	250.3 h	13.2cd	13.9 cd	7.2 a

Means in columns marked with the same letter do not differ significantly at p = 0.05 according to Tukey's multiple test

Table 6. Effect of biopreparations on mycorrhizal frequency (F%) in the roots of Elsanta, Honeoye and Elkat strawberry plants. Analysis of the results, July 2013–2014

Treatment	Mycorrhizal frequency (F%)		
	Elsanta	Honeoye	Elkat
Control	21.1 b	20 b	24.5 b
Control NPK	10.6 a	12.2 a	12.2 a
Micosat F	52.2 e-g	53.4 e-g	47.2 c-e
Humus UP	56.1 gh	58.3 gh	61.1 h
Manure	41.7 cd	40 c	43.4 cd
Vinassa	47.8 d-f	52.8 e-g	55 f-h

Means in columns marked with the same letter do not differ significantly at p = 0.05 according to Tukey's multiple test

Table 7. Effect of biopreparations on the number of spores in the soil collected from under Elsanta, Honeoye and Elkat strawberry plants. Analysis of the results, July 2013–2014

Treatment	Number of spores		
	Elsanta	Honeoye	Elkat
Control	36.3 a	33.7 a	35.3 a
Control NPK	27.7 a	32 a	33.3 a
Micosat F	55 cd	90.7 fg	80.7 f
Humus UP	53.3 cd	83.3 f	100 g
Manure	50.7 c	38 ab	47.7 bc
Vinassa	62.3 de	68.7 e	80.7 f

Means in columns marked with the same letter do not differ significantly at p = 0.05 according to Tukey's multiple test

Assessment of the number of spores of mycorrhizal fungi in rhizosphere soil

Samples of rhizosphere soil, collected in July 2013 and 2014, 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 (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 2003, 2008] (tab. 7).

Statistical analysis

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

RESULTS

The results of the experiments show a positive influence of the applied bioproducts on plant growth and development, the degree of mycorrhizal colonization in the roots and the presence of beneficial soil microorganisms in the rhizosphere of strawberry plants of the cultivars 'Elsanta', 'Honeoye' and 'Elkat' grown under greenhouse conditions.

Assessment of plant growth characteristics

Applications of the bioproducts increased the growth of strawberry plants, the surface area of the leaves and their fresh and dry weight. The use of the biopreparation Humus UP resulted in an increase of plant growth in all the cultivars studied, and in significant increase in leaf surface area and leaf fresh weight in the cultivar 'Elsanta', compared with plants fertilized with NPK. The use of Humus UP also resulted in significant increase in leaf dry weight in the cultivar 'Elsanta'. Compared with the plants fertilized with the biofertilizers, the 0-control plants (not treated with biofertilizers) as well as those fertilized with NPK were characterized by a smaller surface area and lower fresh and dry weight of leaves (tab. 2).

Assessment of root growth characteristics

The biopreparations Humus UP and Vinassa also had a positive influence on the size of the root system. Applications of Humus UP resulted in significant increase in root length and root surface area, and in significant increase in root diameter, compared with NPK and 0-control plants (tab. 3). Under the influence of Vinassa, root volume was significantly increased in all the strawberry cultivars studied, and the number of root tips was significantly enhanced in the cultivar 'Honeoye'. The NPK control plants had a smaller root system, with roots of smaller length, volume and surface area, and with fewer root tips (tab. 4).

Microbiological analysis of substrates

The biopreparations Micosat F and Humus UP, and also bovine manure, contributed to increases in the overall number of bacteria, spore-forming bacteria, and the total number of filamentous fungi in the rhizosphere soil of strawberry plants of the three test cultivars 'Elsanta', 'Honeoye' and 'Elkat' in comparison with NPK fertilization. Micosat F increased significantly the total number of fungi in 'Elkat'; the manure increased significantly the total number of bacteria in 'Elkat', and Humus UP resulted in a doubling of the total number of spore-forming bacteria in 'Elsanta' compared with the control plants fertilized with NPK (tab. 5).

Assessment of root colonization by mycorrhizal arbuscular fungi

The results of microscopic examinations of root specimens showed that the roots of 'Elkat' strawberry plants treated with the biopreparation Humus UP were characterized by the highest degree of mycorrhizal colonization in comparison with the roots of plants fertilized with NPK. Under the influence of Humus UP, the roots of the cultivars 'Elsanta' and 'Honeoye' were also more frequently colonized by mycorrhizal arbuscular fungi than the roots of plants fertilized with NPK and those of 0-control (unfertilized) plants. The results of the examinations showed that the roots of plants fertilized with NPK and the roots of 0-control plants (not treated with NPK or the biopreparations) were colonized by AM fungi to a significantly smaller extent (tab. 6).

Assessment of the number of spores of mycorrhizal fungi in the rhizosphere soil

Soil analyses for the presence of mycorrhizal fungi showed that the use of Humus UP in 'Elkat' strawberry plants resulted in significant increase in the number of spores of AM fungi in the soil in comparison with NPK-fertilized control plants. The preparations Micosat F and Vinassa contributed to a significant increase in the number of spores (table 7). Mineral (NPK) fertilization resulted in a lower number of spores of AM fungi in the rhizosphere soil of strawberry plants.

DISCUSSION

The results of our experiments indicate a positive influence of the applied biopreparations on the growth and development of strawberry plants of the cultivars 'Elsanta', 'Honeoye' and 'Elkat'. The use of the preparation Humus UP had a favourable effect on plant growth characteristics (tab. 2). The obtained results are in agreement with the findings of other authors. Rzepka-Plevens et al. [2011] had studied the influence of auxins and humic acids from three types of mineral soil on the rooting, growth and adaptation to *ex vitro* conditions of two strawberry cultivars ('Elsanta' and 'Senga Sengana') grown *in vitro*. They found that humic acids stimulated the growth of root and shoot biomass of strawberry plants. They also found a beneficial effect of humic acids on plant growth during the period of adaptation in the greenhouse, in particular on the number of leaves, leaf length and width. The application of humic acids also had a beneficial effect on the number of roots and their length, compared with the plants treated with auxins and the control plants. Fan et al. [2011] studied the effects of inoculation with mycorrhizal fungi on root biomass and morphology in plants of three strawberry cultivars ('Kent', 'Jewel' and 'Saint-Pierre') in a greenhouse or phytotron. Regardless of the cultivar tested and the salinity of the soil, inoculation of strawberry plants with *Rhizophagus irregulare* resulted in an increase in fresh and dry weight of shoots compared with non-inoculated control plants. They also observed a positive influence of inoculation on root growth characteristics such as length, surface area, volume, diameter, number of lateral roots, and fresh and dry weight of roots. Grant et al. [2010] investigated the effect of water deficit on the physiological and morphological characteristics of strawberry plants of ten cultivars ('Elsanta', 'Florence', 'Symphony', 'Delia', 'Emily', 'Cambridge Favourite', 'Totem', 'Hapil', 'Elvira' and 'Idea') grown in a polytunnel. They observed that leaf surface area and leaf fresh weight were twice as high in the plants grown under optimal water conditions as in the plants grown under a water deficit.

In the experiments described in this paper, the use of preparations containing mycorrhizal fungi in their composition (Micosat F) improved the growth and development of the roots and shoots of strawberry plants (tabs 2, 3, 4). This corresponds to the experiments conducted by Boyer et al. [2014], who studied the effect of inoculation with two strains of mycorrhizal fungi on the growth and drought tolerance of strawberry plants of the cultivar 'Everest'. The data obtained by them showed that inoculation of roots with mycorrhizal fungi largely contributed to increasing the weight and length of roots in comparison with the non-inoculated control plants. In a greenhouse experiment, Sinclair et al. [2014] studied the effects of colonization by arbuscular mycorrhizal fungi of plants of three strawberry cultivars ('Albion', 'Charlotte' and 'Seascape') growing in saline soil. They found that mycorrhization of roots helped to increase root diameter, surface area, length, volume, and the number of lateral roots in comparison with non-inoculated plants.

In our experiments, applications of the biopreparations Micosat F and Humus UP increased the total number of spore-forming bacteria and microscopic fungi compared with the NPK control plants and non-fertilized plants (tab. 5). This confirms the findings of Ding et al. [2013], who assessed the influence of biofertilizers and rhizosphere bacteria on reducing bacterial wilt in potato under greenhouse conditions. They found that the use of biofertilzers BIO23 and BIO36 increased the overall population of bacteria and actinomycetes, whereas organic fertilization (compost) increased the total number of fungi. Pešaković et al. [2013] studied the influence of biofertilizers (inocula of PGPR 1 and PGPR 2), among other things, on the population of soil microorganisms in the rhizosphere of 'Senga Sengana' strawberry plants under greenhouse conditions. They demonstrated that applications of PGPR 1 bacteria resulted in a 3-fold increase in the total number of microorganisms and in the number of actinomycetes, whereas applications of PGPR 2 resulted in a doubling of the total number of fungi and the total number of bacteria of the genus *Azotobacter* in the soil.

The results obtained by us indicate a beneficial effect of using the biopreparation Humus UP on increasing mycorrhizal frequency in the roots of plants of the three strawberry cultivars studied. In our previous study, the preparations Micosat F and Humus UP had increased 20-fold the degree of mycorrhizal association in the roots of 'Elsanta', compared with the plants fertilized with NPK [Sas Paszt et al. 2011]. Also, Boyer et al. [2014] studied the effects of inoculation with two strains of mycorrhizal fungi (*Funneliformis mosseae* BEG25, *Funneliformis geosporus* BEG11) on the growth and drought tolerance of plants of the strawberry cultivar 'Everest'. They found that with increasing levels of water stress, root colonization by the mycorrhizal fungus of the genus *F. mosseae* also increased. In a greenhouse experiment, Koron et al. [2014] investigated the effects of biofumigation and soil heating, and applications of three types of biopreparations, which included stems of brassica plants (*Brassica juncea, Eruca sativa, Sinapis alba*), on the growth and yielding of 'Marmolada' strawberry plants and colonization of their roots by arbuscular mycorrhizal fungi. The observations showed that biofumigation of strawberry plants with a biopreparation containing in its composition shoots of rucola (*Eruca sativa*) increased the degree of root colonization by AM fungi to the greatest extent. In a greenhouse experiment on the effect of colonization by AM fungi of the roots of three strawberry cultivars ('Albion', 'Charlotte' and 'Seascape') growing under conditions of salinity, Sinclair et al. [2014] found that inoculation of the roots increased the degree of mycorrhizal colonization in the roots of the strawberry cultivars studied compared with the roots of non-inoculated control plants.

Our results show that applications of the bioproducts Humus UP and Micosat F resulted in an increase in the number of spores in the rhizosphere soil compared with the rhizosphere soil of plants fertilized with NPK. Also, our previous studies had proven that the preparations Micosat F and BioFeed Amin contributed to an increase in the number of spores of AM fungi in the rhizosphere of 'Elsanta' strawberry plants [Sas Paszt et al. 2011]. This is confirmed by Garland et al. [2011], who had studied the effects of inoculation with species of mycorrhizal fungi (commercial mixture of AM fungi and *Rhizophagus intraradices*) on the growth and yielding of strawberry plants. Inoculation of plants with AM fungi increased the number of species of spores of these fungi in the rhizosphere of the inoculated plants compared with the rhizosphere of plants growing in the control soil. Malusa et al. [2007] had found that inoculation of strawberry roots with the preparation Micosat F contributed to a significant increase in the number of spores in the rhizosphere of the strawberry cultivars studied.

The activity of beneficial microflora in the rhizosphere is not only one of the factors determining normal growth of plants but also an important potential source of their immunity to infectious diseases [Sas Paszt et al. 2015]. Thanks to rhizosphere bacteria and mycorrhizal fungi, the absorptive surface of plant roots increases, and so does the effectiveness of the uptake by plants of mineral ions, include in phosphorus, potassium, magnesium and other macro- and microelements [Sas Paszt et al. 2011]. Therefore, it is important to investigate the influence of bioproducts on plant growth and development, and on the number and activity of beneficial soil microorganisms.

CONCLUSIONS

1. The applied biopreparations had a positive influence on the growth and development of plants of the strawberry cultivars studied, the degree of mycorrhizal frequency in the roots, and on the number of soil microorganisms, compared with plants fertilized with NPK.

2. Biopreparation Humus UP had the most beneficial effect on the tested characteristics of plant vegetative growth in the three strawberry cultivars studied.

3. Applications of the bioproducts contributed to the greatest extent to a significant increase in root and shoot growth characteristics, and in the number of spore-forming bacteria in the cultivar 'Elsanta'.

4. Under the influence of the applied bioproducts, the cultivar 'Elkat' was characterized by the highest total number of bacteria and filamentous fungi and the largest number of spores of AM fungi in the rhizosphere, as well as the highest degree of mycorrhizal colonization in the roots.

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WPŁYW BIONAWOZÓW NA WZROST ROŚLIN I MIKROBIOLOGIĘ RYZOSFERY ODMIAN TRUSKAWKI UPRAWIANEJ W SZKLARNI

Streszczenie. Celem przeprowadzonych badań była ocena wzrostu i rozwoju trzech odmian roślin truskawki nawożonych bionawozami w warunkach szklarniowych. Doświadczenie przeprowadzono w kompleksie szklarniowym Instytutu Ogrodnictwa w Skierniewicach w latach 2013 i 2014 (w okresie od lutego do lipca). Rośliny trzech odmian truskawki Elsanta, Honeoye i Elkat posadzono w rizoboksach i zastosowano następujące nawożenie: kontrola 0 (bez nawożenia), kontrola NPK, substrat bakteryjno-mikoryzowy Micosat, obornik, Humus UP i Vinassa. Aplikacja Humusu UP miała korzystny wpływ na

wzrost roślin, pole powierzchni liści, ich świeżą i suchą masę, stopień kolonizacji mikoryzowej w korzeniach oraz liczbę spor arbuskularnych grzybów mikoryzowych (AMF) w rizosferze roślin. Biopreparaty Humus UP oraz Vinassa miały również pozytywny wpływ na wielkość systemu korzeniowego, ogólną liczbę bakterii, liczbę bakterii wytwarzających formy przetrwalnikowe i ogólną liczbę grzybów strzępkowych w glebie rizosferowej roślin truskawki, w porównaniu z nawożeniem mineralnym NPK w warunkach szklarniowych.

Słowa kluczowe: bioprodukty, wzrost roślin, grzyby AMF, bakterie rizosferowe, *Fragaria* × *ananasa*

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