THE INFLUENCE OF BIOPRODUCTS ON ROOT GROWTH AND MYCORRHIZAL OCCURRENCE IN THE RHIZOSPHERE OF STRAWBERRY PLANTS 'ELSANTA'

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A B S T R A C T

An experiment was carried out in a greenhouse of the Research Institute of Horticulture (RIH) to evaluate the effect of new organic fertilizers and amendments on root growth and mycorrhizal abundance and species richness in the rhizosphere of strawberry plants cv. 'Elsanta'. The plants were grown in rhizoboxes (sized 37 cm x 1.8 cm x 20 cm), filled with 1.85 kg of a podsolic soil collected from an uncultivated field of an experimental organic orchard of the RIH. The soil characteristics were: pH 5.5, organic matter content 1.5%, P content 51 mg P kg^{-1}, K content 158 mg K kg^{-1}. The plants were treated with different organic fertilizers and amendments: dry granulated bovine manure (Doktor O'grodnik), extract of vermicompost (Humus UP), extract of humates (Humus Active + Aktywit PM), plant extract (BioFeed Amin), extract from several seaweed species reinforced with humic and fulvic acids (BioFeed Quality), a consortium of beneficial soil organisms (Micosat), a stillage from yeast production (Vinassa) and a solution of titanium (Tytanit). Plants treated with BioFeed Amin, BioFeed Quality, Micosat, Vinassa and Tytanit received also half dose of dry manure. A standard NPK fertilization (NPK control) and a not fertilized control were also included. The following parameters were measured: root growth and morphological parameters, number of arbuscular mycorrhizal fungi (AMF) spores, mycorrhizal frequency of AMF in the roots. The chemical composition of the applied products and of soil were also determined.

The treatment inducing the highest development of mycorrhizas in the roots of strawberry plants cv. 'Elsanta' were Micosat and BF Amin. The treatments BF Quality and BF Amin had the most beneficial effect on the formation of AMF spores in the trap cultures.

Application of the bioproducts had a positive effect on root growth parameters in comparison with the plants fertilized with NPK. The use of BF Quality and Humus UP induced a reduction of the amount of mineral nutrients in the soil.

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Key words: arbuscular mycorrhizal fungi, trap cultures, species richness of AMF, mycorrhizal frequency, root growth parameters

INTRODUCTION

Organic farming is considered an important element of the Polish and EU's strategy for the development of the agricultural sector and the production of organic fruits has been increasing in recent years. However, the limited availability of traditional organic fertilizers (i.e. manure), and scarce information about the effects of new kinds of organic fertilizers, such as plant extracts (Sas Paszt et al., 2009) or microbial inocula (Malusá et al., 2007), are serious obstacles threatening the future development of the sector.

Arbuscular mycorrhizal fungi (AMF) of the phylum *Glomeromycota* live in symbiosis with a majority (over 80%) of land plants (Smith and Read, 1997). The symbiosis generally increases the productivity and growth vigour of the plants and their resistance to environmental stresses (Allen, 1991; Błaszkowski, 2003; Głuszek et al., 2008), as well as their resistance to pathogens (Azcón-Aquilar and Barea, 1992). However, there are also some examples of plant growth reduction after mycorrhizal inoculation. Such effect was related to several factors, including carbon drain by the fungus, plant density, soil fertility, soil temperature and agricultural practices (Koide, 1985; Jifon et al., 2002; Schroeder and Janos, 2004; Li et al., 2008; Covacevich and Echeverria, 2009). Application of mycorrhizal inocula can increase species diversity of these fungi in the rhizosphere and consequently improve the growth, yielding and yield quality of cultivated fruit crops (Sas Paszt and Żurawicz, 2005; Sas Paszt and Głuszek, 2007). Establishing the best conditions for plant-fungus symbiosis is the key factor to obtaining efficient mycorrhization (Estaún et al., 1994). The continuous cultivation of one crop reduces the diversity and richness of AMF species in the soil (An et al., 1993).

Research on the influence of arbuscular mycorrhizal fungi on the growth and yield of strawberry plants has been a subject of many papers (Vosatka et al., 1992; Sas Paszt and Żurawicz, 2004; Sas Paszt and Żurawicz, 2005, Derkowska et al., 2008; Yin et al., 2010). The first report on the role of AM fungi in the growth and yielding of strawberry dates back to the early twentieth century (White, 1929), but more papers were published during the second half of that century (Mosse, 1953, 1956; Nemec, 1974). A lot of

those papers concern the mycorrhization of plants propagated *in vitro* with different strains of mycorrhizal fungi (Vestberg, 1992ab; Cassells et al., 1996; Borkowska, 2002; Stewart et al., 2005; Borkowska, 2006). Studies of indigenous mycorrhizal fungi colonizing the roots of strawberry plants have also been conducted (Nemec, 1974; Didier et al., 2003; Derkowska et al., 2008; Botham et al., 2009). Much of the research work has concentrated on the effects of mycorrhizal fungi on the growth and yield characteristics of strawberry plants (Niemi and Vestberg, 1992). Plants of the cultivar 'Elsanta' have not yet been the subject of many studies on their symbiosis with AM fungi (Varma and Schüepp, 1994). Roots of the strawberry cultivar 'Elsanta' readily form symbiotic associations with arbuscular fungi (Varma and Schüepp, 1994). Maintaining species diversity of these fungi in the rhizosphere environment of an organic orchard may have an effect on the growth and health status of the plants and the quality of the fruit they produce. New innovative products and technologies for organic fruit production are under development, including the use of beneficial soil microorganisms and fertilizers or amendments of organic origin (Sas Paszt et al., 2008; Malusà and Sas Paszt, 2009). The influence of organic amendments on the development of mycorrhizal fungi has been reported by several authors. The technologies rely on the use of these bioproducts as they are or in a form enriched with beneficial soil microorganisms (Malusà et al., 2007). Several organic fertilizers and amendments have been recently tested and introduced, which are also acting as natural stimulators of plant growth and development (Chelariu and Ionel, 2005; Gousterova et al., 2008; Khan et al., 2009; Chelariu et al., 2009; Meszka and Bielenin, 2009). These are preparations of natural (plant or animal) origin, harmless to humans and the environment, which contain nutrient elements and biologically active substances (i.e. plant hormone-like substances, enzymes) as well as other compounds that stimulate plant growth, yield, quality or tolerance to abiotic stresses. Enrichment of organic bioproducts with beneficial soil bacteria and mycorrhizal fungi could enhance the effectiveness of the organic products in plant growth stimulation and fruit production.

The study, carried out within the framework of a project aimed at developing new products and technologies for organic fruit production in Poland, evaluated the effect of new organic fertilizers and amendments on root growth and mycorrhizal abundance and biodiversity in the rhizosphere of strawberry plants 'Elsanta'.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse over a 5-month period with the use of frigo-plants of the strawberry cultivar 'Elsanta'. The plants were planted in rhizoboxes (37 x 1.8 x 20 cm), filled with 1.85 kg of a podsolic soil collected from an uncultivated field of an experimental organic orchard of the Research

Institute of Horticulture (Fig. 1). The plants were subjected to the following growing conditions: photoperiod 16/8 h (day/night), light intensity 70 μM m^{-2} s^{-1}, temperature 25/20 °C and air humidity approx. 50% (Dinkelaker et al., 1993; Sas et al., 2003; Sas Paszt and Żurawicz, 2005). The soil content of nutrient elements was: organic matter 1.5%, P 51 mg P kg^{-1}, K 158 mg K kg^{-1}, pH 5.5.

The following experimental treatments were applied:

1) Control (no-treatment) – unfertilized podsolic soil.
2) Standard NPK soil fertilization: 4 g NH_4NO_3 $plant^{-1}$, 3 g triple superphosphate $plant^{-1}$ and 6 g K_2SO_4 $plant^{-1}$.
3) Dry granulated bovine manure, suitable for organic farming (Doktor O'grodnik), containing: 55% C, 1% N, 0.3% P and 1% K; the product contains also micro-elements and soil micro-organisms (1 10^6 CFU g^{-1}). It was applied to the soil, near the root system (1 g $plant^{-1}$), at planting.
4) Micosat (CCS Aosta s.r.l.) – a mixture of beneficial soil fungi and bacteria containing: spores, hyphae and root fragments colonized by five species of AM fungi: *Glomus mosseae* Taxtersensu Gerd. & Trappe, *G. intraradices* Schenk & Smith, *G. caledonium* (Nicolson et Gerdemann) Trappe et Gerdemann, *G. viscosum* Nicolson and *G. coronatur* (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 $plant^{-1}$), at planting. The plants treated with the microbial inoculum received before planting basic soil fertilization (0.5 g $plant^{-1}$) with dry manure (containing 1% N, 0.3% P and 1% K).

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 $plant^{-1}$) and then three times during the growing period (1% solution, 15 ml $plant^{-1}$).
6) Humus Active + Aktywit PM (Ekodarpol) – Humus Active is a soil improver with active humus and a population of beneficial microorganisms containing 0.78% C, 0.03% N, 1050 mg kg^{-1} P and 4119 mg kg^{-1} K. Aktywit PM is a soil improver containing 20.5% C, 0.92% N, 81.2 mg kg^{-1} P and 42990 mg kg^{-1} K. The products were first applied to the soil at planting as a 2% solution (15 ml $plant^{-1}$) and then three times during the growing period (1% solution, 15 ml $plant^{-1}$).
7) BioFeed Quality (Agrobio Products B.V.) – an extract from several seaweed species reinforced with humic and fulvic acids containing 0.6% C, 0.07% N, 32.6 mg kg^{-1} P. The product was applied to the plants five times during the growing period as a 0.5% solution (25 ml

plant^{-1}). The plants treated with the seaweed extract received before planting basic soil fertilization (0.5 g plant^{-1}) with dry manure (containing 1% N, 0.3% P and 1% K).

8) BioFeed Amin (Agrobio Products B.V.) – an extract of 100% vegetal amino-acids containing 1.12% C, 0.14% N, 347 mg kg^{-1} P. The product was applied to the plants five times during the growing period as a 0.5% solution (25 ml plant^{-1}). The plants treated with amino-acids received before planting basic soil fertilization (0.5 g plant^{-1}) with dry manure (containing 1% N, 0.3% P and 1% K).
9) Tytanit (Intermag) – titanium (Ti) 0.8% (5 g Ti in 1 l of working solution), pH 3.40, containing 3163 mg kg^{-1} Ti. The product was applied to the plants five times during the growing period as a 0.5% solution (5 ml plant^{-1}). The plants treated with this bioproduct received before planting basic soil fertilization (0.5 g plant^{-1}) with dry manure (containing 1% N, 0.3% P and 1% K).
10) 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 applied to the plants five times during the growing period as a 0.5% solution (50 ml plant^{-1}). The plants treated with this product received before planting basic soil fertilization (0.5 g plant^{-1}) with dry manure (containing 1% N, 0.3% P and 1% K). The table 1 shows the amount of mineral nutrients applied with the different treatments calculated per one hectare of soil.

Each product was applied to 10 plants grown in 5 rhizoboxes (2 plants per rhizobox) (Fig. 1) placed in a completely randomized design.

Table 1. Amounts of mineral elements applied to the plants in rhizoboxes with the different bioproducts, expressed per hectare of soil (kg)

Treatment	**N**	**P**	**K**
Control	0.0	0.0	0.0
NPK	70	26	100
Manure	45	13	17
Micosat	23	6.5	12
Humus UP	1	0.1	0.2
Humus Active	0.5	0.1	2
BF Quality + ½ manure	23	6.5	8.5
BF Amin + ½ manure	23	6.5	8.5
Tytanit + manure	45	13	17
Vinassa + ½ manure	23	6.5	8.5

Figure 1. The set up of the greenhouse experiment with strawberry plants grown in rhizoboxes

Determination of root growth and morphological parameters

At the end of the growth period, root dry weight was determined in accordance with the analytical procedure developed by Ostrowska et al. (1991). Root morphological parameters (total root length, root diameter, root surface area, root volume and total number of root tips) were measured by an image analysis system with an Epson scanner, controlled by WinRhizo software (Regent Instruments Inc.).

Determination of soil chemical parameters

The amount of N and C in the soil was determined with the Dumas method using a TruSpec CNS analyzer. Available P and K was determined with the Egner-Rhiem method of emission spectrometry (Cygański, 1997). The electrochemical method in KCl was used to measure soil pH.

Determination of mycorrhizal frequency

In order to calculate mycorrhizal frequency, roots were cold-stained using the Phillips and Hayman method (1970) and modified by Turnau et al. (2001). In particular, bleaching with 10% KOH and acidification with 5% lactic acid were carried out for 24 hours each. Staining was performed with 0.01% aniline blue (Sumorok et al., 2008).

The microscopic analysis of the roots was carried out according to Trouvelot's method (1986). Thirty 1-cm-long root segments were selected randomly from each of the stained samples. The segments were examined under a Nikon Eclipse E200 microscope. The mycorrhizal frequency (F%) and mycorrhizal intensity (both relative – M%, and absolute – m%) were assessed in each root segment. The mycorrhizal parameters were calculated using the Mycocalc software

(http://www2.dijon.inra.fr.mychintec/Mycocalc-pgr/download.html).

For each experimental treatment three replicates were analyzed, constituting in total 90 root segments (Derkowska et al., 2008).

Identification of spores

In order to identify the spores of arbuscular mycorrhizal fungi, trap cultures were set up with narrowleaf plantain (*Plantago lanceolata* L.). The plants were planted in 0.5 l pots filled with a mixture of rhizosphere soil (obtained from the root zone of strawberry plants grown in rhizoboxes) and autoclaved sand, at a ratio of 1:1 v/v (Błaszkowski, 2003). The pots were placed in SunBags (Sigma) at an air humidity of approx. 70%. After a six-month growing period 200g samples of the pot substrate were taken from the trap cultures combinations, and spores were isolated by wet sieving and centrifuging in a sucrose gradient (at 20% and then at 60%) (Brundrett et al., 1996). The isolated spores were used to prepare microscopic specimens. The spores were picked with a preparation needle or an automatic pipette and placed on a Petri dish, where they were divided into morphotypes according to size, shape, and colour of spores. These parameters together with the number and thickness of layers of spore walls were assessed for identification purposes (Błaszkowski, 2003, 2008). The shape and size of spores were determined on at least 50 intact spores mounted in a drop of water or lactic acid placed on a microscope slide. The dimensions were determined using a light microscope equipped with an ocular micrometer. The thickness of layers of spore walls and germination walls was measured in spores freshly isolated and crushed in PVLG or PVLG+Melzer's reagent (1:1, v/v), and observed under a light microscope equipped with a micrometer eyepiece (Błaszkowski, 2003). The observed AMF species were named according to Schüßler et al. (2001) and Błaszkowski (2003).

The results were statistically evaluated by analysis of variance. Comparisons of means were done at $p \leq 0.05$ with the Duncan test.

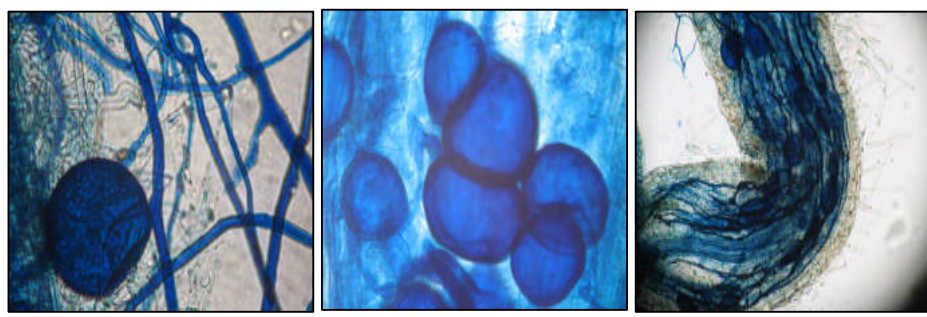
RESULTS

The highest values of mycorrhizal frequency and relative intensity were recorded in the roots of the plants inoculated with the microbial consortium (Micosat), followed by the plants from the treatments with: BF Amin + manure and Humus UP (Tab. 2). Lower values of mycorrhizal frequency were recorded in the roots of the plants treated with Vinassa + manure, Humus Active + Aktywit PM and the no-treatment control. The lowest value of mycorrhizal frequency was determined for the NPK standard fertilization (Tab. 2). Treatment of strawberry plants with bioproducts increased formation of mycorrhizal structures in the roots (Fig. 2).

The highest dry weight of roots was recorded in the treatment with Tytanit + manure, followed by Manure and Micosat (Tab. 3). The plants fertilized with NPK had the lowest dry weight of roots. The largest surface

Table 2. Mycorrhizal colonization parameters determined in the roots of strawberry plants 'Elsanta' grown in rhizoboxes. The details of the experimental treatments are given in the materials and methods. (Means of 90 replicates; different letters referring to the same parameter indicate statistically significant difference $p \leq 0.05$)

Experimental treatments	F [%]	M [%]	m [%]
Control	11.11 ab	0.11 a	1.0 ab
NPK	2.22 a	0.02 a	0.67 a
anure	3.33 a	0.03 a	1.00 ab
Micosat	45.55 d	1.63 b	3.33 bc
Humus UP	21.11 bc	0.48 a	2.37 b
Humus Active + Aktywit PM	15.56 b	0.70 a	4.00c
BF Quality + ½ manure	3.33 a	0.03 a	1.00 a
BF Amin + ½ manure	28.89 c	1.67 b	6.74 d
Tytanit + Manure	3.33 a	0.08 a	0.78 a
Vinassa + ½ manure	16.67 b	0.26 a	1.74 ab



A **B** **C**

Figure 2. Mycorrhizal structures in the roots of strawberry plants cv. 'Elsanta': A – Mycorrhizal mycelium and spores in the roots of the plants treated with Micosat (mag. 10 x 40), B – Vesicles in the roots of the plants treated with Humus Activ + Aktywit PM (mag. 10 x 40), C – Root fragment with mycelium and vesicles from plants treated with Humus UP (mag. 10 x 10)

area was found in the roots of the plants treated with Vinassa + manure, followed by the Control, Tytanit + manure, and Humus Active with Aktywit PM. The smallest surface area of roots was recorded after the application of Manure (Tab. 3). Root diameter ranged from 0.60 to 0.66 mm, with the largest value found in the plants treated with NPK, Humus

Table 3. Root growth and morphological parameters of strawberry plants cv. 'Elsanta' treated with different bioproducts (means of 3 replicates; different letters referring to the same parameters indicate statistically significant differences $p \leq 0.05$)

Treatment	Root dry weight [g]	Root surface area [cm^2]	Root diameter [mm]	Root volume [cm^3]	Root length [cm]	Number of root tips
Control	2.2 bc	726 ef	0.64 b	11.93 d	3582.49 bc	6723.5 d
NPK	0.93 a	574 d	0.66 c	4.54 a	1348.82 a	2787.7 a
Manure	2.7 d	277 a	0.64 b	9.85 c	3007.95 b	5609.8 bc
Micosat	2.7 d	608 cd	0.61 a	10.19 c	3323.66 b	6236.2 c
Humus UP	2.5 cd	648 e	0.62 ab	9.17 c	2945.50 b	6202.7 c
Humus Active + Aktywit PM	1.6 ab	578 d	0.66 c	8.00 b	2297.18 ab	4388.3 b
BF Quality + ½ manure	1.7 b	479 bc	0.60 a	9.28 c	3198.24 b	6978.0 d
BF Amin + ½ manure	1.9 b	609 cd	0.61 a	10.14 c	3323.34 b	6708.4 d
Tytanit + manure	3.2 e	649 e	0.62 ab	12.16 d	4087.93 c	9028.2 e
Vinassa + ½ manure	2.4 c	802 f	0.67 c	9.56 c	3582.49 bc	5298.8 bc

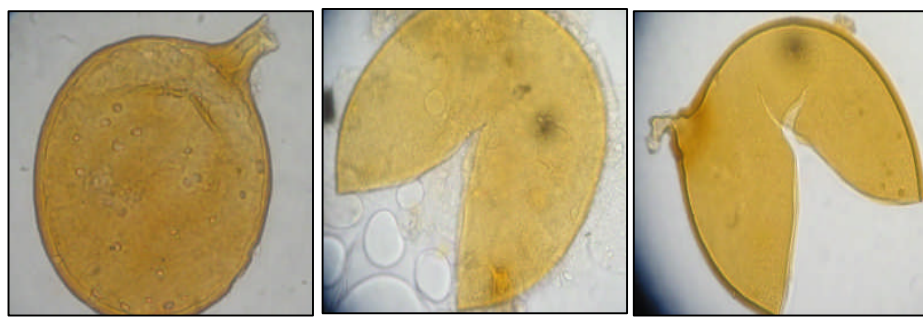
Active + Aktywit PM and Vinassa (Tab. 3). The plants not fertilized and those treated with Tytanit + manure showed the largest volume of roots, while those treated with NPK had the lowest (Tab. 3). The highest induction of elongation of roots was produced by Tytanit + manure; all other treatments except the NPK, also favoured to some extent elongation of roots (Tab. 3). Consequently, Tytanit showed the highest numbers of root tips, also in this case, all organic products increased the branching of roots, while this parameter was not influenced by NPK treatment (Tab. 3).

In comparison to the control (without any treatment), BF Quality + manure, BF Amin + manure, Micosat, Humus Active + Activit PM, and manure induced the formation of the highest number of AMF spores in the trap cultures containing the rhizosphere soil of strawberry plants 'Elsanta' grown in rhizoboxes (Tab. 4). The lowest average and total numbers of spores were recorded for the treatments Vinassa + manure, NPK, and the no-treatment control (Tab. 4).

Several AM fungi species, ranging from 3 to 6, were found in the trap cultures containing rhizospheric soil from the diverse treatments (Tab. 5, Fig. 3). The most common species of AM fungi found in the experimental combinations were: *Glomus claroideum* (found in all 10 treatments), *Scutellospora dipurpurescens* (found in 9 treatments), *G. mosseae* (identified in 7 treatments), *G. fasciculatum* (present in 6 treatments). *Glomus caledonium* was found in three treatments, and

Table 4. Average number of spores isolated from trap cultures containing the rhizosphere soil of strawberry plants cv. 'Elsanta' grown in rhizoboxes (means of 3 replicates; different letters indicate statistically significant differences between the applied treatments $p \leq 0.05$)

Treatment	Mean number of spores per sample
Control	101.97 a
NPK	117.42 ab
Manure	150.66 b
Micosat	160.04 b
Humus UP	120.56 ab
Humus Active + Aktywit PM	155.47 b
BF Quality + ½ manure	199.32 b
BF Amin + ½ manure	173.53 b
Tytanit + manure	108.14 ab
Vinassa + ½ manure	89.68 a



Glomus constrictum *Glomus claroideum* *Glomus mosseae*

Figure 3. Spores of arbuscular mycorrhizal fungi found in the trap cultures containing rhizospheric soil of strawberry plants cv. 'Elsanta' grown in rhizoboxes (mag. 10 x 40)

G. macrocarpum in two. On the other hand, four AMF species were found in only one treatment: *Glomus micro-aggregatum* and *G. constrictum* were present only with Manure, *G. pallidum* with Vinassa, and *G. drummondii* in the NPK control (Tab. 5).

The highest amount of all macro-elements in the rhizosphere soil was determined for the NPK treatment (Tab. 6). No differences were found in the content of C and N and in the pH. The soil from the NPK treatment showed the significantly highest content of P and K, while among the other bioproducts the differences were much smaller, even though statistically significant (Tab. 6).

Table 5. Arbuscular mycorrhizal fungi isolated from pot trap cultures established with *Plantago lanceolata* (n=3). Details of the treatments are given in the materials and methods

Experimental combinations	Species richness
Control	*Glomus claroideum* N.C. Schenck & G.S. Sm. *Glomus macrocarpum* Tul. & C. Tul. *Glomus mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe *Scutellospora dipurpurescens* J.B. Morton & Koske
NPK	*Glomus claroideum* N.C. Schenck & G.S. Sm. *Glomus drummondii* Blaszk. & C. Renker *Glomus fasciculatum* (Thaxt.) Gerd. & Trappe *Glomus mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe *Scutellospora dipurpurescens* J.B. Morton & Koske
Manure	*Glomus claroideum* N.C. Schenck & G.S. Sm. *Glomus constrictum* Trappe *Glomus microaggregatum* Koske, Gemma & P.D. Olesia *Glomus mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe *Scutellospora dipurpurescens* J.B. Morton & Koske
Micosat	*Glomus claroideum* N.C. Schenck & G.S. Sm. *Glomus fasciculatum* (Thaxt.) Gerd. & Trappe *Glomus mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe *Scutellospora dipurpurescens* J.B. Morton & Koske
Humus UP	*Glomus claroideum* N.C. Schenck & G.S. Sm. *Glomus mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe *Scutellospora dipurpurescens* J.B. Morton & Koske
Humus Active +Aktywit PM	*Glomus claroideum* N.C. Schenck & G.S. Sm. *Glomus fasciculatum* (Thaxt.) Gerd. & Trappe *Scutellospora dipurpurescens* J.B. Morton & Koske
BF Quality+ ½ manure	*Glomus claroideum* N.C. Schenck & G.S. Sm. *Glomus fasciculatum* (Thaxt.) Gerd. & Trappe *Glomus macrocarpum* Tul. & C. Tul. *Glomus mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe *Scutellospora dipurpurescens* J.B. Morton & Koske
BF Amin+ ½ manure	*Glomus caledonium* (T.H. Nicolson & Gerd.) Trappe & Gerd. *Glomus claroideum* N.C. Schenck & G.S. Sm. *Glomus fasciculatum* (Thaxt.) Gerd. & Trappe *Glomus mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe
Tytanit + manure	*Glomus caledonium* (T.H. Nicolson & Gerd.) Trappe & Gerd. *Glomus claroideum* N.C. Schenck & G.S. Sm. *Scutellospora dipurpurescens* J.B. Morton & Koske
Vinassa + ½ manure	*Glomus caledonium* (T.H. Nicolson & Gerd.) Trappe & Gerd. *Glomus claroideum* N.C. Schenck & G.S. Sm. *Glomus fasciculatum* (Thaxt.) Gerd. & Trappe *Glomus mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe *Glomus pallidum* I.R. Hall *Scutellospora dipurpurescens* J.B. Morton & Koske

Table 6. Carbon, nitrogen, phosphorus, potassium and pH level in the rhizosphere soil after treatment with different bioproducts (n=3; different letters referring to the same nutrient or pH level indicate statistically significant differences $p \leq 0.05$)

Treatment	C [%]	N [%]	P [mg·100g^{-1}]	K [mg·100g^{-1}]	pH in KCl
1. Control	1.06 a	0.10 a	6.11 b	8.63 c	5.9 a
2. NPK	1.09 a	0.10 a	12.1 d	22.2 f	5.1 a
3. Manure	1.06 a	0.09 a	5.67 a	6.45 a	5.6 a
4. Micosat	1.10 a	0.10 a	5.68 a	9.75 d	5.8 a
5. Humus UP	1.08 a	0.09 a	6.07 b	6.56 a	5.7 a
6. Humus Active + Aktywit PM	1.03 a	0.09 a	5.60 a	9.79 d	5.8 a
7. BF Quality +½ manure	1.06 a	0.09 a	6.18 bc	7.71 b	5.7 a
8. BF Amin +½ manure	1.10 a	0.10 a	6.18 bc	8.89 c	5.7 a
9. Tytanit + manure	1.05 a	0.10 a	6.33 c	11.1 e	5.6 a
10. Vinassa +½ manure	1.08 a	0.09 a	6.43 c	9.12 d	5.9 a

DISCUSSION

The highest mycorrhizal frequency (45.55) was achieved following the application of the microbial consortium Micosat. Such result supports findings showing the efficacy of inoculation of plants with AMF (Jeffries et al., 2003; Malusá et al., 2007). The inoculation of strawberry plants with Micosat also resulted in an increased number of spores produced by the fungi. However, only one species *Glomus mosseae* of the four present in the inoculum was found as a spore. This species was also among the indigenous fungi present in the podsolic soil, used for all of the experimental treatments. This fact could be due to the differences in either sporulation or various ability of the fungal species to inoculate the plant roots.

However, the plants treated with BF Quality (a seaweed extract) had one of the lowest mycorrhizal frequency rate (3.33%), whereas the plants treated with BF Amin (a plant extract enriched with humic acids) had a higher mycorrhizal frequency rate (28.89%).

The low level of mycorrhizal frequency in the roots observed in this study was related to the growing period (winter time) and the specific conditions of growth in greenhouse rhizoboxes. Indeed, our previous results revealed that the mycorrhizal frequency in strawberry roots of the plants grown in the greenhouse is much lower than in the roots of filed grown plants (Sas Paszt et al., unpublished data). Our earlier data clearly indicate, that the highest level of mycorrhizal frequency in the roots is found in July-August and the

frequency declines during the growing season (Derkowska et al., 2008). The micropropagated strawberry plants of 'Senga Sengana' grown in January-February have a generally lower mycorrhizal frequency than the plants cultivated in May-June (Niemi and Vestberg, 1992). It has been found that the mycorrhizal frequency in asparagus roots of the plants grown in greenhouse conditions was much lower than in the roots of the field grown plants (Pedersen et al., 1991). According to our previous experience and the literature data in the field, mycorrhizal frequencies in strawberry roots are often below 50%, which indicates that strawberry roots are generally characterized by lower levels of mycorrhizal colonization (Vestberg, 1992ab; Vosatka et al., 1992; Williams et al., 1992) than other plant species (Jurkiewicz et al., 2010).

The largest number of spores in the trap cultures, containing rhizospheric soil of strawberry plants and sand, was obtained after the combined applications of manure and plant-derived preparations: BF Quality or BF Amin (obtained from processed seaweed or herbaceous plants). The results obtained show a favourable impact of the organic products on the sporulation of AM fungi. Results of other studies confirm the positive effect of seaweed-derived substances (Kuwada et al., 2005; Kuwada et al., 2006a,b) and other compounds of plant origin (Poulin et al., 1993; Ishii et al., 1997; Gryndler and Hršelová, 1998; Gryndler et al., 2005; Horii et al., 2009) on the development of AMF.

In our study, several spores of the identified AMF species were collected in the trap cultures made with a mixture of rhizosphere soil and sand (1:1). Indeed, up to six AMF fungal species were identified, according to the morphological features.

All bioproducts had a positive effect on the growth parameters of the roots, particularly in comparison to NPK. Interestingly, these effects were found also from the treatments with bioproducts applied to the leaves (Vinassa and Tytanit). Effect of foliar fertilization on root growth and morphology was already noted (Malusá et al., 2007). Interestingly, Tytanit, which is supposed to enhance the photosynthetic metabolism, induced the highest root elongation and also number of root tips. Previous experiments with preparations containing titanium concerned primarily the impact of this element on the size and quality of the yield produced by various fruit plants (Serrano et al., 2004), including strawberry (Laszlovszky-Zmarlicka, 2006; Skupień and Oszmiański, 2007ab; Michalski, 2008), and also on the chemical composition of plants (Borkowski et al., 2007; Wallace et al., 1977). The positive effect of the biofertilizer Vinassa, which is the stillage resulting from the processing of sugar beet molasses in the production of yeast, on the development of the root system of strawberry plants is confirming the data from the other studies (Chelariu and Ionel, 2005; Chelariu et al., 2009). Vestberg (1992ab) evaluated suitability of *Glomus mosseae* and also *G. intraradices* for inoculation of

strawberry and reported that the latter was found to be the most efficient fungus species as it increased shoot growth several-fold.

Generally, the organic fertilizers induced a considerable branching of the root system, as derived from the high total root length and number of tips. On the contrary, the NPK treatment was always causing the smallest root system, which is consistent with the observations of the other authors (Haynes and Goh, 1987; Sas et al., 2003; Glinicki, verbal communication, 2011). This feature is common in chemically fertilized plants and it is considered also a possible factor affecting the plant ability to tolerate environmental stresses (e.g. water deficiency).

We found that in the rhizosphere of strawberry plants 'Elsanta' fertilized with various bioproducts there were spores of arbuscular mycorrhizal fungi of several species of the genus *Glomus* and also of the fungus *Scutellospora dipurpurescens*. However, their occurrence was not uniform. For example, *Glomus claroideum*, a species frequently found in Polish sandy soils (Błaszkowski, 2003), was present in all the experimental treatments. The *Glomus mosseae*, a very common species found also in the rhizosphere of strawberry plants (Didier et al., 2003), was present in soil from several treatments. This species is also used as inoculum to increase the resistance of strawberry plants to stress caused by excess phosphorus (Stewart et al., 2005) and drought (Yin et al., 2010). Four other species, namely *Glomus microaggregatum*, *G. constrictum*, *G. pallidum* and *G. drummondii*, were found in the soil treated with only one bioproduct. This difference in the presence of AMF species in rhizospheric soil could be related to the effect of the treatment. Indeed, the plant is actively interacting with the soil to promote the establishment of mycorrhizal symbiosis for nutritional purposes (Hartmann et al., 2009). Changes in its physiology caused by differences in availability of nutrients could thus modify the chemical communication between the plant and the different AMF species, leading to a selective establishment of the symbiosis (Allen et al., 2003). Such hypothesis could be supported by findings from several authors. *Glomus macrocarpum* had also been recorded by Didier et al. (2003) in trap cultures of the rhizosphere soil of strawberry plants, but they found no spores of this species in soil samples from strawberry field crops. *Glomus caledonium*, identified in the rhizosphere of the strawberry plants fertilized with Tytanit, was found to occur in intensively and semi-intensively cultivated agricultural soils (Oehl et al., 2003). In our study, the trap cultures were used to multiply, isolate and identify AMF species naturally colonizing strawberry roots. The species richness was rather narrow, when compared with the results of other studies done in natural environment. Błaszkowski and Czerniawska (2011) identified more than 30 AMF species of spores isolated both from dune soil and trap cultures established with dune soil.

The fertilizers, amendments and biostimulators used in the experiment influenced, to a varying degree, root growth, the development of AMF species and the structures produced by them. The development of strawberry roots was stimulated by organic amendments and reduced by NPK fertilization. These results are confirmed by other studies, where the application of N fertilizer alone (or in combination with an organic fertilizer) decreased root growth parameters, in comparison with the control or organic fertilizers (Wang et al., 2009). Other studies have revealed that the positive effect of two kinds of vermicomposts on growth of strawberry plants is not influenced only by macronutrients availability, but also by the impact of plant growth regulators produced by microorganisms during the composting process (Wang et al., 2002; Arancon et al., 2004). The soil microorganisms and plant growth regulators in plant extracts (cytokinins, auxins and betaines) can also positively influence plant growth parameters in strawberry plants (Blunden et al., 1997; Esitken et al., 2010). The beneficial impact of organic matter and AMF inocula on plant growth was influenced by organic matter decomposition and nutrient mobilization from organic matter (Hodge et al., 2001; Ravnskov et al., 2006). The different treatments affected to a very limited extent the content of nutrients in the rhizospheric soil, with the exception of the NPK application. Very high amount of N, P and K was found in the soil treated with standard NPK fertilizers. Considering the good growth of the whole plant (data not shown), and in particular of the root system, observed in all treatments, it appears that the applied amount of NPK fertilizers is not fully used by the plants, eventually leading to a reduced microbiological soil biodiversity. The more developed root system of the plants treated with the bioproducts could be associated to a greater efficiency in nutrient uptake and acquisition (data not shown).

CONCLUSIONS

1. The bioproducts BF Quality and BF Amin had the most beneficial effect on the formation of AMF spores in the trap cultures containing rhizospheric soil of strawberry plants cv. ‘Elsanta’.
2. Differences in AMF species population present in the trap cultures could derive from the effects of the applied bioproducts on the plant physiology.
3. The microbial inoculum Micosat and the plant extract BF Amin were the most favourable treatments for the development of mycorrhizas in the roots of strawberry plants cv. ‘Elsanta’.
4. Application of all bioproducts contributed to the increase in root growth parameters, in comparison with NPK fertilized plants.
5. The different bioproducts only slightly affected the content of nutrients in the rhizospheric soil, with the exception of the NPK treatment.

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WPŁYW BIOPRODUKTÓW NA WZROST KORZENI I WYSTĘPOWANIE MIKORYZ W RIZOSFERZE ROŚLIN TRUSKAWKI ‘ELSANTA’

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STRESZCZENIE

Doświadczenie prowadzono w kompleksie szklarniowym Instytutu Ogrodnictwa przez 5 miesięcy z użyciem sadzonek frigo truskawki odmiany ‘Elsanta’. Rośliny posadzono w rizoboksach o wymiarach 37 cm x 1.8 cm x 20 cm, wypełnionych glebą bielicową (1,85 kg) pochodzącą z nieuprawianych poletek z Ekologicznego Sadu Doświadczalnego IO (pH 5.5, zawartość materii organicznej – 1,5%, zawartość P – 51 mg P kg^{-1}, zawartość K – 158 mg K kg^{-1}). Rośliny nawożono różnymi nawozami organicznymi i dodatkami – suchym granulowanym obornikiem bydlęcym (Doktor

O'grodnik), ekstraktem z wermikompostu (Humus UP), ekstraktem kwasów huminowych (Humus Activ + Aktywit PM), ekstraktem z roślin (BioFeed Amin), ekstraktem z roślin wodnych wzbogaconych kwasami huminowymi i fulwowymi (BioFeed Quality), mieszaniną pożytecznych mikroorganizmów glebowych (Micosat), produktem odpadowym z produkcji drożdży piekarniczych (Vinassa), biostymulatorem Tytanit. Wszystkie bioprodukty uzupełniano obornikiem, który był źródłem azotu. Kontrole stanowiły rośliny nawożone NPK oraz nienawożone. Badano następujące parametry: cechy wzrostu i morfologii korzeni, liczbę spor arbuskularnych grzybów mikoryzowych (AMF), stopień frekwencji mikoryzowej grzybów AMF w korzeniach, skład chemiczny zastosowanych produktów i gleby.

Bioprodukty Micosat i BF Amin + obornik w największym stopniu wpłynęły na zasiedlanie korzeni roślin truskawki odmiany 'Elsanta' przez arbuskularne grzyby mikoryzowe. Nawożenie bioproduktami BF Quality i BF Amin miało istotny wpływ na formowanie spor grzybów AMF w kulturach pułapkowych, założonych z gleby rizosferowej badanych roślin truskawki.

Zastosowane bioprodukty miały korzystny wpływ na cechy wzrostu korzeni w porównaniu z roślinami nawożonymi NPK. Aplikacja BF Quality i Humus UP wpłynęła na zmniejszenie zawartości składników mineralnych w glebie.

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Słowa kluczowe: arbuskularne grzyby mikoryzowe, kultury pułapkowe, bogactwo gatunkowe grzybów AMF, frekwencja mikoryzowa, cechy wzrostu korzeni