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EFFECT OF BIOLOGICALLY ACTIVE TOTALHUMUS® AND BACTERBASE ON THE GROWTH *ex vitro* OF STRAWBERRY, BLUEBERRY AND HIP ROSE MICROCUTTINGS

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ABSTRACT

One of the key steps in plant micropropagation is rooting and acclimatization of microcuttings. The aim of the study was to investigate the suitability of commercial biopreparations, TotalHumus® and Bacterbase, to stimulate the growth of young fruit plants derived from *in vitro* propagation. TotalHumus® is made from brown coal. Bacterbase is a bacterial preparation containing *Bacillus velezensis* and *Bacillus amyloliqefaciens* (Skierniewickie Microorganisms) with antifungal properties that stimulates the growth and yielding of plants. Unrooted microshoots of strawberry 'Grandarosa' and highbush blueberry 'Chandler', and rooted *in vitro* microcuttings of hip rose 'Konstancin' were planted in a peat substrate. Three weeks after planting *ex vitro*, the plants were treated with the biopreparations. Four times, at two-week intervals, the plants were drenched and simultaneously sprayed with mineral fertilizer 0.2% Hydrovit (control), 0.04% TotalHumus® and 0.03% Bacterbase, which were used separately or in combinations. In hip rose and strawberry, compared to the control, similar or better growth parameters of shoots and roots were observed after the use of TotalHumus® and/or Bacterbase. The plants were characterized by the highest fresh weight, longer shoots/runners and more shoots than in the control (mineral fertilization). In strawberry, root parameters were significantly improved by TotalHumus®, and in rose by Bacterbase. The use of both TotalHumus® and Bacterbase separately or in combination significantly reduced the occurrence of symptoms of rose leaf infection with powdery mildew. The biopreparations had no effect on highbush blueberry.

Key words: biologically active preparations, *ex vitro* acclimatisation, *Fragaria* × *ananassa*, *Vaccinium corymbosum*, *Rosa rugosa* × *R. beggeriana*

INTRODUCTION

One of the key steps in the micropropagation of plants is rooting and acclimatization of microcuttings [Nowak 1998, Vestberg et al. 2002]. In particular, the acclimatization of young plants propagated *in vitro* requires very careful treatments: planting in the socalled sterile substrates such as mineral wool or peat substrate, maintaining high humidity and relatively low light intensity, and then gradual hardening of plants to lower humidity, higher light intensity and variable temperature [Chandra et al. 2010]. Plants derived from sterile *in vitro* conditions are easily infected by various pathogens. It is therefore necessary to ensure adequate chemical protection. However, due to Regulations (EC) No 1107/2009 of the European Parliament and

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Council [2009], the permitted chemical protection is very limited. The safety of workers is also very important. Therefore, it is necessary to use treatments that are friendly to the environment and people. For many years, researchers have been paying attention to the socalled biotization of microcuttings during plant acclimatization to *ex vitro* conditions [Nowak 1998, Vestberg et al. 2002, Gosal et al. 2010, Orlikowska et al. 2017, Kanani et al. 2020, Trzewik et al. 2020c]. The biotization process is the inoculation of microcuttings either *in vitro* or young plants *ex vitro* at acclimatization stage with beneficial microorganisms, e.g. symbiotic bacteria or mycorrhizal fungi [Nowak 1998, Vestberg et al. 2002, Gosal et al. 2010, Sas-Paszt et al. 2020]. Plant growth promoting microorganisms (PGPM) are applied in order to stimulate growth of young plants, especially to improve the development of the root system, as well as to enhance their resistance to biotic and abiotic stress factors [Nowak 1998, Chandra et al. 2010]. Some of the microorganisms, such as *Trichoderma harzianum*, *Bacillus amyloliquefaciens*, *Gliocladium virens* and *Serendipita indica* are used as biological protection agents against many fungal diseases [Abdullah et al. 2008, Ji et al. 2013, Chowdhury et al. 2015, Paraszkiewicz et al. 2017, Trzewik et al. 2020a, b].

Agricultural biostimulants include diverse formulations of compounds that are applied to plants or soils to regulate and enhance the crop's physiological processes, thus making them more efficient [Sas-Paszt et al. 2019]. In order to improve the rooting efficiency and acclimatization of *in vitro* propagated plants, there is a growing interest in the use of growth biostimulants. Biostimulants are preparations of organic (plant or animal) origin, friendly to people and the environment [Canellas et al. 2015, Sas-Paszt et al. 2015, 2019, 2020]. They are produced on the basis of natural plant extracts and beneficial microorganisms (bacteria, microscopic fungi and mycorrhizal fungi). This paper presents the results on the effects on plant growth of two biologically active preparations: TotalHumus® and Bacterbase. TotalHumus® is made of brown coal and contains over 90% of dead organic matter. It is rich in humic acids, humins and fulvic acids, which help to restore the appropriate microflora, thus limiting the development of pathogens and eliminating toxic substances. Bacterbase is a bacterial preparation for stimulating the growth and yielding of plants in field crops and greenhouses. Bacterbase is a preparation characterized by an exceptionally high survival rate of beneficial organisms contained in it; it contains strains of Skierniewickie Microorganisms, the species *Bacillus velezensis* and *Bacillus amyloliquefaciens*. Antifungal properties of these *Bacillus* sp. have been confirmed by numerous studies [Nam et al. 2009, Cai et al. 2017, Fan et al. 2018, Myo et al. 2019, Sas-Paszt et al. 2020].

The aim of the research was to investigate the suitability of commercial biopreparations, TotalHumus® and Bacterbase, to stimulate the growth of young strawberry, highbush blueberry and hip rose plants derived from *in vitro* propagation. TotalHumus® and Bacterbase preparations were applied during the microcutting acclimatization stage and in the early growth of young plants. Previous studies have shown that humic acids and other Skierniewickie Microorganisms (MS) stimulate the growth of vegetable, ornamental, and fruit plants [Sas-Paszt et al. 2015, 2020, Trzciński et al. 2018].

MATERIAL AND METHODS

Strawberry (*Fragaria* × *ananassa* Duchesne) 'Grandarosa', highbush blueberry (*Vaccinium corymbosum* L.) 'Chandler' and hip rose (*Rosa rugosa* × *R. beggeriana*) 'Konstancin' microcuttings were used for the research. The plants were propagated *in vitro* according to the standard methods: strawberry [Wojtania 2020], highbush blueberry [Wojtania et al. 2019] and hip rose [Wojtania and Matysiak 2018]. The hip rose microcuttings were rooted *in vitro* [Wojtania and Matysiak 2018], and then planted in a peat substrate (Alonet Substrat pH 5.5–6.5) in multicell propagation trays (multiplates with 5 × 5 cm cells). The strawberry and highbush blueberry microcuttings were rooted directly in the peat substrates: strawberry – Alonet pH 5.5–6.5; highbush blueberry – peat moss (Baltic Peat pH 3.6–4.2), in multiplates (5 × 5 cm). Due to their vigorous growth, the rose and strawberry plants were transplanted into the same kind of substrate they were grown in before, but in P9 pots, the blueberry plants remained in the multiplates. During the acclimatization and early growth, the plants were grown in plastic mini-greenhouses (58 × 40 × 22 cm)

with two adjustable 'dial' ventilators, in a growing chamber at a temperature of 23°C, under the light of LED lamps (50–60 µmol m^{-2} s^{-1}). Three weeks after planting *ex vitro*, the plants were drenched and simultaneously sprayed with the fertilizer and/or preparations: (A) control, i.e. standard mineral fertilization with 0.2% Hydrovit (Hydrokomplet s.c., Nowa Wieś, Częstochowa, Poland; (B) 0.04% TotalHumus® (THE Sp. z o.o., Kłodawa, Poland); (C) 0.03% Bacterbase (THE Sp. z o.o., Kłodawa, Poland); (D) 0.04% TotalHumus® + 0.03% Bacterbase (in one solution); (E) 0.2% Hydrovit + 0.04% TotalHumus® + 0.03% Bacterbase. The plants were treated with the above-mentioned agents every 2 weeks, starting from July 10, then on July 24, August 7, and August 21, 2020. Immediately after the last treatment, the plants were transferred to the greenhouse. There were 30 plants of each species in each combination.

Observations and measurements were made 25 days after the last treatment and concerned the following parameters: length of shoots or runners, number of shoots/runners, fresh mass of shoots, dry mass of shoots, leaf area, chlorophyll index (CCI), fluorescence, root system parameters, degree of natural leaf infection by powdery mildew (rose) and degree of root colonization by arbuscular mycorrhizal fungi.

The analyses of leaf area and root system parameters were performed using an Epson Expression 10000 XL root scanner (Regent Instruments Company, Canada). The aerial part of each plant was placed on a tray and then scanned. The root system was placed on a sieve and cleared of soil by gentle washing. The roots were weighed and then scanned using the above-mentioned scanner. The growth characteristics of the plants (leaf area, root length, root area, root diameter, root volume and number of root tips) were determined using the WinRhizo software.

Chlorophyll index was measured using a CCM-200 chlorophyll content meter (OptiSciences Int., Hudson NH, USA), and **fluorescence** with MINI PAM fluorometer (Walz, Fehraltorf, Switzerland).

Degree of leaf infection by powdery mildew (rose) was evaluated according to the disease index [Wojdyła 2019]: 0 – no symptoms, 1 – up to 1% of leaf surface covered with mycelium, 2 – from 1.1 to 5%, 3 – from 5.1 to 10%, 4 – from 10.1 to 20%, 5 – more than 20% of leaf surface covered with mycelium. The measurements were performed 2.5 months after the last treatment.

Degree of root colonization by arbuscular mycorrhizal fungi was assessed as follows. Fragments of the root system of rose and strawberry plants (10 g of each replicate) taken from the experiment were stained according to the method developed by Derkowska et al. [2015], and blueberry roots according to the method of Phillips and Hayman [1970]. Then, microscopic preparations were made, which were analyzed using a Nikon 50i microscope (objectives with magnification: 20×, 40×, 60×, 100×), and photographic documentation of the observed mycorrhizal structures was made. The degree of root colonization by arbuscular mycorrhizal fungi was assessed using the method of Trouvelot et al. [1986]. On the basis of the obtained results, the mycorrhizal frequency (F%), mycorrhizal intensity (m%, M%) and arbuscule abundance (a%, A%) were calculated using the MYCOCALC computer program, available on the website: https://www2.dijon.inrae.fr/mychintec/Mycocalc-prg/download.html.

Statistical analysis

All the parameters were analysed with one-way ANOVA design. All the calculations were done with the Statistica package (StatSoft v. 13.1). The means were compared by Duncan's test at $p = 0.05$. Measurements of the length of the longest shoot or runner, and number of shoots/runners per plant were made for 30 plants as replications. The degree of natural leaf infection by powdery mildew (rose) was evaluated for 25 plants; assessment according to the above-described disease index was performed independently by 3 persons; ANOVA analysis was performed on three means obtained from the assessment of each of the three persons. Five plants (five replications) were used for estimations of the fresh mass of shoots, dry mass of shoots, area of all the leaves per plant, root system parameters, and degree of root colonization by arbuscular mycorrhizal fungi. For CCI and fluorescence, five plants were evaluated for three leaves each, with three replicate measurements; ANOVA was performed on five means obtained for each plant.

RESULTS

Strawberry. In young plants, the best results were obtained with the application of 0.04% TotalHumus®, with the largest plant height and leaf number (Tabs. 1 and 2, Fig. 1). After application of TotalHumus® or Bacterbase, the plants were distinguished by a significantly higher runner number, by ca. 30%, compared to other treatments. The plants treated with TotalHumus® showed also a slightly higher (statistically insignificant) fresh and dry mass, leaf area and CCI. After using TotalHumus® alone or in combination with Bacterbase, their root system displayed a considerably larger area by ca. 30–50%, and volume by ca. 35%, and the roots were thicker by ca. 80–90% compared to the treatment with Hydrovit (control) (Tab. 3). The highest number of the root tips (2486.2) was observed in the control and this value was approximately three to four times higher compared to other treatments.

Highbush blueberry. The biopreparations tested poorly influenced the growth of young plants of high bush blueberry. Compared to the control, slightly (statistically insignificant) longer shoots, higher the dry mass of shoots, chlorophyll index, root volume and root tip number were observed after the application of TotalHumus® alone and/or combined with Bacterbase (Tab. 4–6, Fig. 1). The treatment with TotalHumus® however, significantly increased root diameter compared to the control (Tab. 6). Generally, Bacterbase had a neutral or negative effect on the growth parameters of highbush blueberry plants.

Hip rose. The best growth parameters of shoots and leaves were observed after the combined use of 0.04% TotalHumus® and 0.03% Bacterbase. In this treatment, significantly higher dry mass and leaf area were recorded (Tab. 7 and 8, Fig. 1). The use of both TotalHumus® and Bacterbase separately or in combination significantly reduced the occurrence of symptoms of rose leaf infection with powdery mildew (Tab. 8, Figs. 2 and 3). The disease index was 2–3 times lower with these treatments (0.76–1.17) compared to the control (2.34). The best developed root system was obtained with 0.03% Bacterbase (Tab. 9). Compared to the control, the root length, root area and root volume were increased by ca. 40%, 70% and 100%, respectively.

Table 1. Effect of biostimulants on the growth of shoots and runners of strawberry 'Grandarosa'

Treatment	Plant height (cm)	Fresh mass (g)	Dry mass (g)	Runner number	Runner length (cm)
A. Hydrovit 0.2%	9.3 ±0.6 ab	8.5 ±2.5 a	0.89 ±0.24 a	2.8 ±1.1 b	25.6 ±9.2 a
B. TotalHumus® 0.04%	10.7 ±1.5 a	10.7 ±1.5 a	1.20 ±0.14 a	3.6 ±0.7 a	26.3 ±8.3 a
C. Bacterbase 0.03%	9.1 ±1.0 b	8.4 ±2.4 a	0.87 ±0.28 a	3.3 ±1.0 a	27.6 ±7.0 a
D. TotalHumus® 0.04%+Bacterbase 0.03%	9.5 ±1.2 ab	9.6 ±2.9 a	0.88 ±0.34 a	2.4 ±1.2 b	19.7 ±11.1 b
E. Hydrovit + TotalHumus® + Bacterbase	9.2 ±0.8 b	10.3 ±3.1 a	0.92 ±0.29 a	2.7 ±1.2 b	17.5 ±6.5b

Means separation within columns by Duncan's test at a significance level $p = 0.05$; means marked with the same letter do not differ significantly

Table 2. Effect of biostimulants on the morphological features of 'Grandarosa' strawberry leaves

Treatment	Leaf number	Leaf area (cm^2)	Chlorophyll index (CCI)	Fluorescence (Fv/Fm)
A. Hydrovit 0.2%	8.3 ±0.8 ab	431.6 ±85.0 a	19.9 ±1.8 ab	0.82 ±0.02 a
B. TotalHumus® 0.04%	8.6 ±0.6 a	497.2 ±89.7 a	22.9 ±3.2 a	0.82 ±0.01 a
C. Bacterbase 0.03%	8.3 ±1.3 ab	424.8 ±93.4 a	18.7 ±2.3 b	0.82 ±0.02 a
D. TotalHumus® 0.04% + Bacterbase 0.03%	7.4 ±0.8 b	424.7 ±49.4 a	20.1 ±0.9 ab	0.82 ±0.02 a
E. Hydrovit + TotalHumus® + Bacterbase	7.8 ±0.6 ab	412.5 ±79.2 a	21.3 ±3.6 ab	0.82 ±0.03 a

Explanations – see Table 1

Table 3. Effect of biostimulants on the growth characteristics of 'Grandarosa' strawberry roots

Treatment	Root length (cm)	Root area (cm^2)	Root diameter (mm)	Root volume (cm^3)	Root tip number
A. Hydrovit 0.2%	371.4 ±107.2 a	55.7 ±15.5 b	0.48 ±0.04 b	0.67 ±0.20 b	2486.2 ±650.1 a
B. TotalHumus® 0.04%	405.1 ±90.3 a	82.1 ±17.3 a	0.65 ±0.07 a	1.30 ±0.34 a	581.8 ±40.3 b
C. Bacterbase 0.03%	318.4 ±44.9 ab	55.0 ±10.7 b	0.55 ±0.8 ab	0.77 ±0.21 b	828.6 ±154.2 b
D. TotalHumus® 0.04% + Bacterbase 0.03%	373.9 ±80..9 a	74.2 ±13.1 a	0.64 ±0.10 a	1.20 ±0.31 a	658.4 ±138.4 b
E. Hydrovit + TotalHumus® + Bacterbase	263.5 ±21.0 b	54.0 ±7.3 b	0.60 ±0.06 a	0.82 ±0.17 b	641.8 ±188.5 b

Explanations – see Table 1

Table 4. Effect of biostimulants on the growth of 'Chandler' blueberry shoots

Treatment	Shoot length (cm)	Shoot number	Fresh mass (g)	Dry mass (g)
A. Hydrovit 0.2%	7.2 ±2.8 ab	3.5 ±1.7 a	0.9 ±0.2 a	0.17 ±0.15 ab
B. TotalHumus® 0.04%	7.6 ±2.4 a	3.5 ±1.5 a	0.9 ±0.5 a	0.23 ±0.10 a
C. Bacterbase 0.03%	6.3 ±1.6 b	3.3 ±1.3 a	0.6 ±0.3 a	0.07 ±0.04 b
D. TotalHumus® 0.04% + Bacterbase 0.03%	7.1 ±1.5 ab	3.8 ±1.4 a	1.1 ±0.4 a	0.16 ±0.06 ab
E. Hydrovit + TotalHumus® + Bacterbase	7.3 ±2.0 a	2.6 ±1.2 b	0.9 ±0.3 a	0.11 ±0.03 ab

Explanations – see Table 1

Table 5. Effect of biostimulants on the morphological features of 'Chandler' blueberry leaves

Treatment	Leaf area (cm^2)	Chlorophyll index (CCI)	Fluorescence (Fv/Fm)
A. Hydrovit 0.2%	95.1 ±30.1 a	14.3 ±1.1 ab	0.78 ±0.02 a
B. TotalHumus® 0.04%	95.2 ±33.9 a	14.9 ±3.6 ab	0.77 ±0.01 a
C. Bacterbase 0.03%	50.3 ±31.7 b	12.8 ±2.5 b	0.76 ±0.02 a
D. TotalHumus® 0.04% + Bacterbase 0.03%	96.1 ±30.8 a	17.2 ±1.4 a	0.76 ±0.05 a
E. Hydrovit + TotalHumus® + Bacterbase	64.6 ±22.8 ab	14.9 ±2.5 ab	0.77 ±0.01 a

Explanations – see Table 1

Table 6. Effect of biostimulants on the growth characteristics of 'Chandler' blueberry roots

Treatment	Root length (cm)	Root area (cm^2)	Root diameter (mm)	Root volume (cm^3)	Root tip number
A. Hydrovit 0.2%	33.3 ±10.2 a	6.1 ±1.71 a	0.59 ±0.04 b	0.09 ±0.02 a	121.8 ±45.1 ab
B. TotalHumus® 0.04%	26.3 ±15.3 a	6.0 ±3.5 a	0.75 ±0.11 a	0.11 ±0.06 a	146.6 ±69.6 a
C. Bacterbase 0.03%	18.1 ±9.6 b	4.1 ±1.6 ab	0.77 ±0.12 a	0.07 ±0.02 ab	93.8 ±93.8 ab
D. TotalHumus® 0.04% + Bacterbase 0.03%	18.0 ±3.6 b	4.0 ±1.1 ab	0.70 ±0.09 ab	0.07 ±0.03 ab	89.2 ±89.2 ab
E. Hydrovit + TotalHumus® + Bacterbase	10.9 ±2.7 b	2.2 ±0.8 b	0.64 ±0.11 ab	0.04 ±0.02 b	57.6 ±57.6 b

Explanations – see Table 1

The analyses showed that the application of TotalHumus® in strawberry plants increased the population of arbuscular mycorrhizal fungi compared to Hydrovit fertilization (Tab. 10, Figs. 4 and 5). The use of both biopreparations together in the cultivation of young blueberry plants increased the formation of mycelium coils belonging to the ericoid mycorrhiza, compared to mineral fertilization (Fig. 6). In young rose plants derived from *in vitro* cultures, application of both preparations increased the degree of root colonization by arbuscular mycorrhizal fungi (AGM), compared to the plants treated with this biostimulant, including TotalHumus® and Hydrovit (Tab. 10, Figs. 7 and 8). The analyses show that both biopreparations had

Table 7. Effect of biostimulants on the growth of 'Konstancin' hip rose shoots

Treatment	Shoot length (cm)	Shoot number	Fresh mass (g)	Dry mass (g)
A. Hydrovit 0.2%	13.9 ±5.7 a	1.4 ±0.7 ab	3.3 ±1.8 ab	0.38 ±0.27 c
B. TotalHumus® 0.04%	11.4 ±3.5 b	1.8 ±1.0 a	2.9 ±0.5 ab	0.36 ±0.21 c
C. Bacterbase 0.03%	14.1 ±5.2 a	1.7 ±1.0 ab	3.4 ±0.5 ab	0.21 ±0.15 c
D. TotalHumus® 0.04% + Bacterbase 0.03%	14.2 ±5.4 a	1.7 ±0.9 ab	4.5 ±2.0 a	0.85 ±0.51 a
E. Hydrovit + TotalHumus® + Bacterbase	14.7 ±4.6 a	1.3 ±0.7 b	2.4 ±1.1 b	0.46 ±0.23 bc

Explanations – see Table 1

Table 8. Effect of biostimulants on the morphological features of 'Konstancin' hip rose leaves and leaf infection by powdery mildew

Treatment	Leaf area (cm^2)	Chlorophyll index (CCI)	Fluorescence (Fv/Fm)	Disease index for powdery mildew (1–5)
A. Hydrovit 0.2%	216.2 ±96.3 b	21.3 ±4.8 a	0.79 ±0.05 a	2.34 ±0.24 a
B. TotalHumus® 0.04%	212.9 ±52.5 b	22.0 ±3.8 a	0.81 ±0.02 a	1.17 ±0.28 b
C. Bacterbase 0.03%	219.4 ±41.5 b	21.7 ±3.1 a	0.79 ±0.04 a	0.76 ±0.02 b
D. TotalHumus® 0.04% + Bacterbase 0.03%	345.1 ±161.9 a	25.7 ±3.7 a	0.80 ±0.03 a	1.00 ±0.14 b
E. Hydrovit + TotalHumus® + Bacterbase	193.8 ±24.1 b	24.4 ±3.1 a	0.80 ±0.02 a	1.05 ±0.38 b

Means separation within columns by Duncan's test at a significance level $p = 0.05$; means marked with the same letter do not differ significantly. Disease severity index: 0 – no symptoms, 1 – up to 1% of leaf surface covered with mycelium, 2 – from 1.1 to 5%, 3 – from 5.1 to 10% , 4 – from 10.1 to 20%, 5 – more than 20% of leaf surface covered with mycelium

Table 9. Effect of biostimulants on the growth characteristics of 'Konstancin' hip rose roots

Treatment	Root length (cm)	Root area (cm^2)	Root diameter (mm)	Root volume (cm^3)	Root tip number
A. Hydrovit 0.2%	226.1 ±74.6 b	26.1 ±9.1 b	0.37 ±0.09 a	0.25 ±0.13 b	1275.0 ±729.9 a
B. TotalHumus® 0.04%	165.7 ±48.5 b	20.7 ±6.3 b	0.40 ±0.02 a	0.21 ±0.07 b	968.2 ±225.7 a
C. Bacterbase 0.03%	324.8 ±50.2 a	44.9 ±9.7 a	0.44 ±0.08 a	0.51 ±0.18 a	882.6 ±480.1 a
D. TotalHumus® 0.04% + Bacterbase 0.03%	198.0 ±33.8 b	26.9 ±4.1 b	0.44 ±0.08 a	0.30 ±0.08 b	790.8 ±691.8 a
E. Hydrovit + TotalHumus® + Bacterbase	152.5 ±59.3 b	19.1 ±7.1 b	0.40 ±0.06 a	0.19 ±0.09 b	787.4 ±511.3 a

Explanations – see Table 1

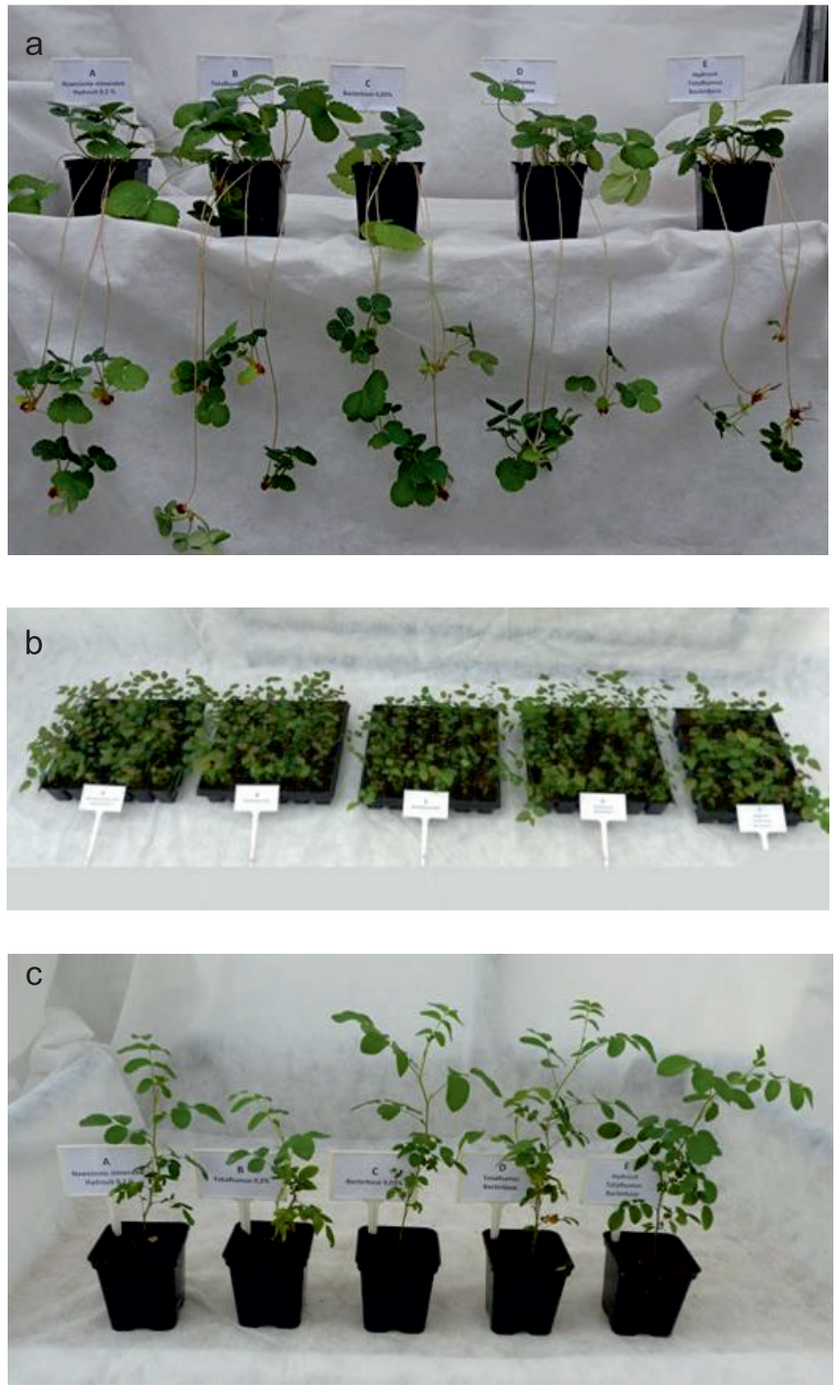


Fig. 1. Plants from *in vitro* cultures, from the top: a – strawberry 'Grandarosa' , b – highbush blueberry 'Chandler' and c – hip rose 'Konstancin', three months after planting *ex vitro*; plants were treated with biostimulants 4 times, every 2 weeks by drenching and simultaneous spraying, starting from the third week after planting *ex vitro*; the following fertilizers/biostimulants were applied (from the left in each photo): A – standard mineral fertilization – 0.1% Hydrovit, B – 0.04% TotalHumus®, C – 0.3% Bacterbase, D – 0.04% TotalHumus® + 0.3% Bacterbase) (in one solution), E – 0.2% Hydrovit + 0.04% TotalHumus® + 0.3% Bacterbase (in one solution)

Fig. 2. Symptoms of powdery mildew (*Podosphaera pannosa*) on leaf of rose 'Konstancin' after natural infection. Disease severity index: 0 – no symptoms, 1 – upto 1% of leaf surface covered with mycelium, 2 – from 1.1 to 5%, 3 – from 5.1 to 10%, 4 – from 10.1 to 20%, 5 – more than 20% of leaf surface covered with mycelium

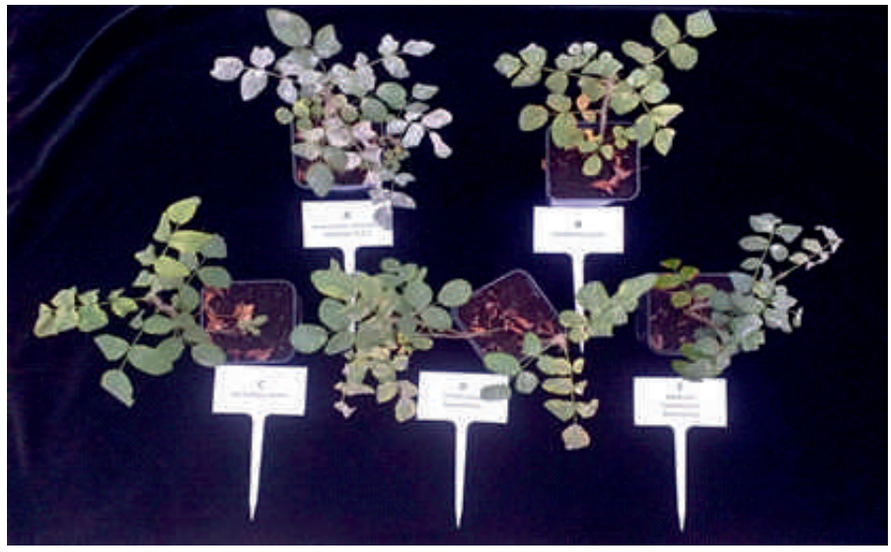


Fig. 3. Degree of plant infection with powdery mildew of rose 'Konstancin', 4 months after planting *ex vitro* depending on treatment with biostimulants (B–E) compared to control (A); A – control – plants not treated with biostimulants but fertilized with standard mineral fertilizer 0.2% Hydrovit, B – 0.04% TotaHumus, C – 0.03% Bacterbase, D – 0.04% TotalHumus® + 0.03%, Bacterbase, E – 0.2% Hydrovit + 0.04% TotalHumus® + 0.03% Bacterbase

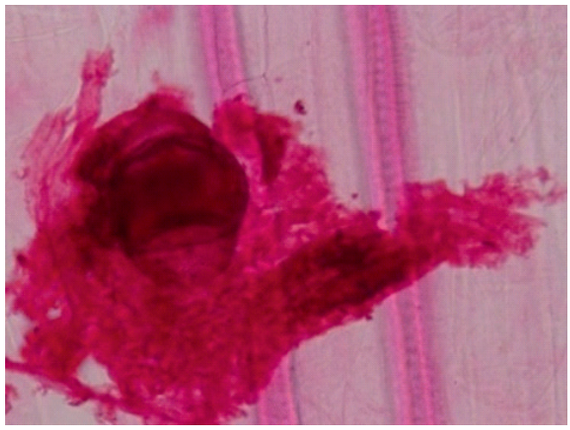


Fig. 4. Spore of arbuscular mycorrhizal fungi in strawberry roots treated with TotalHumus®

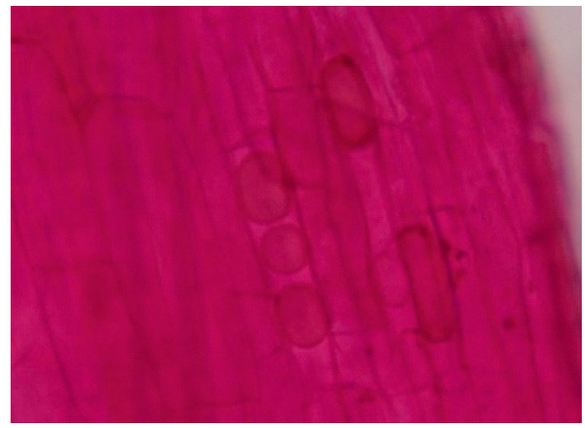
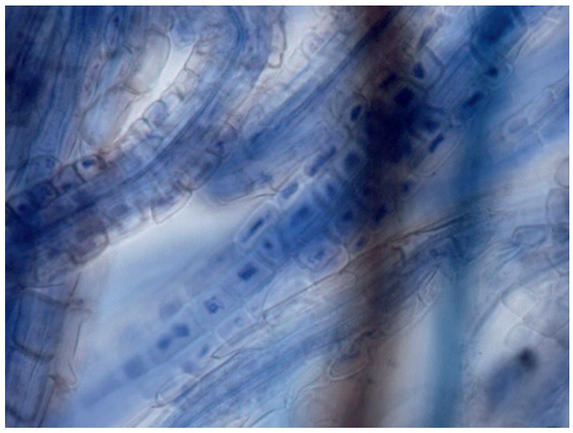


Fig. 5. Vesicular arbuscular mycorrhiza in strawberry roots treated with TotalHumus®, including Bacterbase and Hydrovit



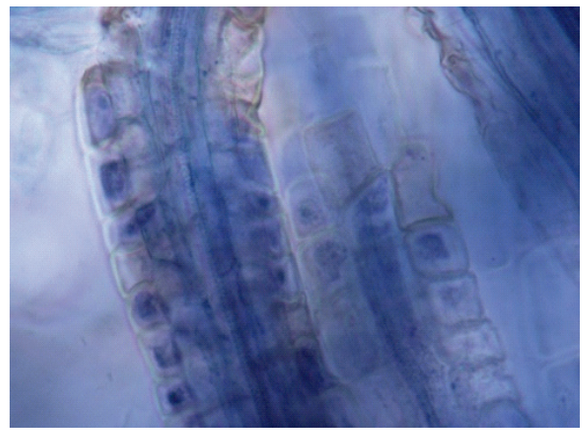


Fig. 6. Coils of ericoid mycorrhiza in blueberry roots treated simultaneously with TotalHumus® and Bacterbase

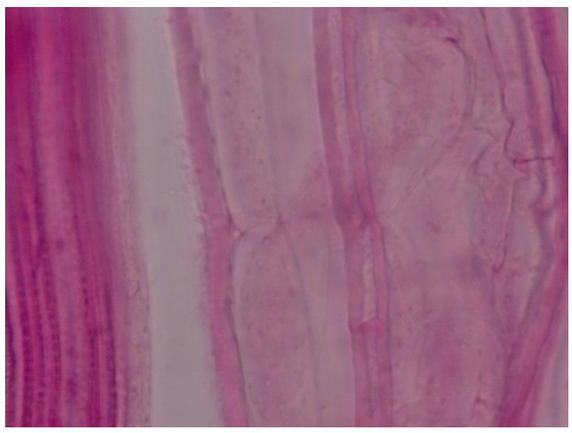


Fig. 7. Mycelium of arbuscular mycorrhizal fungi in rose roots treated with Bacterbase

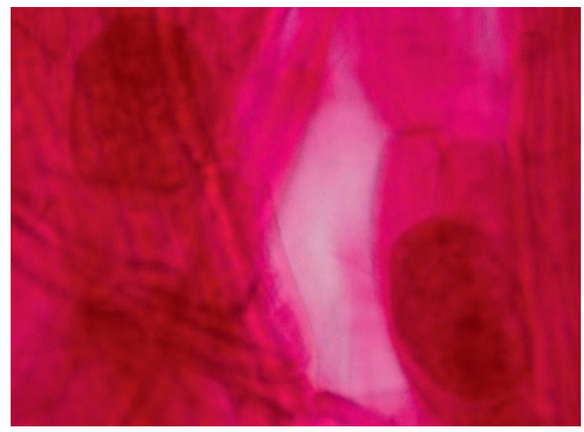


Fig. 8. Vesicular arbuscular mycorrhiza in rose roots treated with Bacterbase

Table 10. Effect of treating rose, strawberry, and blueberry plants with TotalHumus® and Bacterbase biopreparations on the mycorrhizal frequency F% and mycorrhizal intensity m%, M%

Treatment	Strawberry			Highbush blueberry			Rose		
	F%	M%	m%	F%	M%	m%	F%	M%	m%
A. Hydrovit 0.2% (control)	6.7 b	0.47 a	5.33 a	6.7 b	0.25 b	3.89 b	18.9 ab	1.7 ab	8.9 a
B. TotalHumus® 0.04%	16.7 a	1.35 a	8.27 a	8.9 b	1.14 ab	13.17 a	13.3 bc	1.2 ab	9.9 a
C. Bacterbase 0.03%	11.1 ab	0.89 a	7.86 a	11.1 ab	0.56 b	4.64	22.2 a	1.8 a	8.2 a
D. TotalHumus® 0.04% + Bacterbase 0.03%	11.1 ab	0.89 a	7.86 a	23.3 a	1.88 a	7.93 ab	20.0 a	1.5 ab	7.5 a
E. Hydrovit + TotalHumus® + Bacterbase	16.7 a	1.35 a	8.27 a	12.2 ab	0.94 ab	7.58 ab	10.0 c	0.6 b	6.0 a

a positive effect on the presence of mycorrhizal fungi in the roots of the tested plant species. Only the application of mineral fertilization showed the lowest values of the degree of mycorrhizal frequency F% and mycorrhizal intensity m%, M% in the roots of the strawberry and blueberry plants (Tab. 10).

DISCUSSION

In our study, the beneficial effects of TotalHumus® and Bacterbase biostimulants on the growth of young plants derived from in vitro propagation was observed in the case of hip rose and strawberry. Numerous scientific studies show that intensive fertilization with mineral fertilizers does not always have a positive effect on plant growth [Sas-Paszt et al. 2020]. The cited authors reported that applying the microbiological preparations, including a mixture of beneficial bacteria containing three strains (*Bacillus* sp., *Bacillus amyloliquefaciens* and *Paenibacillus*), mineral fertilization can be markedly reduced [Sas-Paszt et al 2015].

The TotalHumus® biostimulant produced from brown coal contains humic acids, humins and fulvic acids. These substances increase the water capacity of the soil, improve the soil structure and increase its microbiological activity, influencing better nutrient uptake by plants [Ulukan 2008, Nardii et al. 2017, Yang et al. 2021]. The direct stimulating effect of humic acids is through changes in plant metabolism. It increases the permeability of cell membranes, accelerating the transport of mineral compounds in the plant [Chen et al. 2004]. Increasing the amounts of mineral compounds increases the intensity of cellular respiration and enhances the processes of cell division. The young plants derived from in vitro propagation are sensitive to ex vitro conditions, react more strongly to the humic acids, compared to older plants, because in young plants, intensive processes of cell division and transport of essential minerals to metabolically active sites take place. Therefore, the application of humic and fulvic acids to young plants is more effective compared to the application in the later stages of development. Moreover, several reports indicate that foliar application of humic acids is more effective than application to the soil [Pettit 2004]. Both humic and fulvic acids work as inhibi-tors of the enzyme that breaks down indole-3-acetic acid (IAA) regulating plant growth and development [Nardi et al. 2002].

Our results showed that both biostimulants had a positive effect on the presence of mycorrhizal fungi in the roots of the studied plant species. Similarly, other bioproducts based on humus-like substances such as Humus UP and Humus Active (Ekodarpol, Poland) had the greatest positive effect on the colonization of strawberry by arbuscular fungi (AMF). The results of microscopic examinations of root specimens conducted by Derkowska et al. [2015] showed that the roots of 'Elkat' strawberry plants treated with the biopreparation Humus UP were characterized by the highest degree of mycorrhizal association in com-

parison with the roots of plants fertilized with NPK. Under the influence of Humus UP, the roots of the strawberry 'Elsanta' and 'Honeoye' were also more frequently colonized by mycorrhizal arbuscular fungi than the roots of plants fertilized with NPK and those of control (unfertilized) plants. The results of the examinations showed that the roots of plants fertilized with NPK and the roots of control plants (not treated with NPK or the biopreparations) were colonized by AM fungi to a significantly smaller extent. A study by Sas-Paszt et al. [2015] showed that the highest dry weight of roots was obtained in the strawberry plants treated with Humus UP, while the lowest was induced by NPK. Among the soil fertilizers, the application of Micosat and Humus UP promoted a good development of the root system with plants showing the highest surface area, volume, total length and number of root tips. In turn, Trzciński et al. [2018] showed that applications of various commercial products containing plant growth promoting microorganisms (PGPM) such as EmFarma Plus (Probiotic, Poland) and/or the Skierniewickie Microoraganisms (MS) containing *Klebsiella oxytoca*, *Pseudomonas fluorescens* and *Pseudomonas* sp. resulted in a significant increase in total populations of diazotrophs in the rhizosphere of carrot, parsley and potato.

Our results clearly showed that the applications of TotalHumus® and Bacterbase, used alone or in combination, significantly reduced the occurrence of symptoms of rose leaf infection with powdery mildew. The biostimulant Bacterbase, containing *Bacillus amyloliquefaciens* and *B. velezensis*, was confirmed to stimulate plant growth and for use as biocontrol agent (own study – data not presented). The antifungal activity of these *Bacillus species* had been confirmed previously by many authors. *Bacillus amyloliquefaciens* strain B94 suppressed *Rhizoctonia solani* and other fungal pathogens in laboratory tests [Yu et al. 2002]. Its antifungal activity was ascribed to the production of an iturin, a cyclic lipopeptide [Yu et al. 2002, Ji et al. 2013]. Ji et al. [2013] reported that *B. amyloliquefaciens* isolate CNU114001 showed broad spectrum activity against 12 phytopathogenic fungi (e.g. *Alternaria panax*, *Botrytis cinerea*, *Colletotrichum orbiculare*, *Penicillium digitatum*, *Pyricularia grisea* and *Sclerotinia sclerotiorum*) in the dual culture method of laboratory tests. These authors reported that the antifungal compound, iturin, isolated from this particular Bacillus species exhibited strong activity against cucumber scleotiorum rot in the laboratory and against tomato grey mould caused by *Botrytis cinerea* and cucumber powdery mildew caused by *Podosphaera xanthii*.

The mechanism of antifungal activity of *B. amyloliquefaciens* was extensively reviewed by Chowdhury et al. [2015]. The authors reported that several results obtained with *B. amyloliquefaciens* FZB42 and other isolates of this species indicated that the mechanism was based on the stimulation of plant immunity rather than through a direct anti-fungal action. Thus, bacterial metabolites, such as surfactin and volatiles, stimulate in plants a defence response such as induced systemic resistance (ISR). However, the role of antimicrobial second-ary metabolites in reducing pathogens in the rhizosphere is of minor importance.

Several strains of *Bacillus velezensis* and closely related *B. amyloliquefaciens* are plantgrowth promoting and biocontrol rhizobacteria [Chowdhury et al. 2015, Dunlap et al. 2016, Fan et al. 2018]. Fan et al. [2018], based on an extensive revision, stated that antifungal compounds produced by these *Bacillus strains* inhibited the growth of pathogens and can also stimulate induced systemic resistance (ISR) in plants. It has been found that besides secondary metabolites also volatile organic compounds are involved in the biocontrol effect exerted by the FZB42 strain under biotic and abiotic stress factors.

Applications of *B. velezensis* strains BS87 and RK1 for pre-plant root-dip application at the concentration of 105 and 106 CFU/ml were found highly effective, showing longterm antifungal activity against *Fusarium oxysporum* f. sp. *fragariae* in strawberry [Nam et al. 2009].

CONCLUSIONS

1. The beneficial effects of TotalHumus® and Bacterbase biostimulants on young plants derived from *in vitro* propagation during the acclimatization stage and early cultivation in a greenhouse were observed in the case of hip rose and strawberry. However, the effect of these biostimulants on the growth of young highbush blueberry plants was insignificant, and in the case of Bacterbase, it was unfavourable.

2. Applications of TotalHumus® and Bacterbase, used alone or in combination, significantly reduced the occurrence of symptoms of rose leaf infection with powdery mildew.

3. In hip rose, the best developed root system was obtained after application of Bacterbase.

4. Both biostimulants had a positive effect on the presence of mycorrhizal fungi in the roots of the studied plant species.

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