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Microbiological Biostimulants in the Improvement of Extended Storage Quality of In Vitro-Derived Plants of Popular Ornamental Perennials

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Abstract: In vitro propagation is a crucial method for the mass production of high-quality plants, but the impact of microbiological interventions during ex vitro storage remains an underexplored aspect. This study aims to assess the effects of three commercial biostimulants in the form of microbiological preparations—BactoFungiStop, AzotoPower, and Guard—applied over six months through foliar sprays on the post-storage quality of *Brunnera macrophylla* 'Silver Heart', *Echinacea purpurea* 'Secret Glow', *Heuchera* × *hybrida* 'Northern Exposure Red', *Persicaria amplecicaulis* 'JS Caliente', and *Rudbeckia* × *hybrida* 'Sunbeckia Sophia Yellow' plants. The monthly application of microbiological preparations adhered to the concentrations recommended by producers. Post-storage evaluations included shoot and root parameters, leaf morphology, and chlorophyll biosynthesis. All microbiological preparations positively influenced shoot elongation in *B. macrophylla* 'Silver Heart'. The microbiological treatments stimulated root development in this species, i.e., increased root length, area, volume, and the number of root forks and tips. In *E. purpurea* 'Secret Glow', all three preparations enhanced shoot length, leaf parameters, and root traits, with Guard demonstrating the highest efficacy. As for *P. amplecicaulis* 'JS Caliente', BactoFungiStop negatively affected shoot and leaf parameters but promoted root development. *Heuchera* × *hybrida* 'Northern Exposure Red' exhibited increased shoot and leaf dimensions with all microbiological treatments, while *Rudbeckia* × *hybrida* 'Sunbeckia Sophia Yellow' displayed positive responses in shoot-related traits but no impact on root development. None of the microbiological preparations influenced chlorophyll biosynthesis in any of the studied species. The results of our research can be implemented in the large-scale production of ornamental plants.

Keywords: *Brunnera macrophylla* (J. F. Adams) I. M. Johnst.; *Echinacea purpurea* (L.) Moench; *Heuchera* × *hybrida*; *Persicaria amplecicaulis* (D.Don) Ronse Decr.; *Rudbeckia* × *hybrida*; acclimatization; foliar sprays; plant growth; root development

Citation: Miler, N.; Tymoszuk, A.; Woźny, A.; Michalik, T.; Wiśniewska, J.; Kulus, D. Microbiological Biostimulants in the Improvement of Extended Storage Quality of In Vitro-Derived Plants of Popular Ornamental Perennials. *Agronomy* **2024**, *14*, 289. https://doi.org/10.3390/agronomy14020289

Academic Editor: Alessandro Miceli

Received: 5 January 2024
Revised: 23 January 2024
Accepted: 25 January 2024
Published: 27 January 2024

1. Introduction

Perennials play a pivotal role in the horticultural market due to their multifaceted significance and enduring appeal. Perennial plants offer a wide array of advantages that contribute to their prominence in the industry. Their longevity reduces the need for frequent replanting, saving both time and resources [1]. Moreover, their diversity in colors, shapes, and sizes makes perennials highly desirable for landscape and garden designs. Beyond their aesthetic value, perennials are essential for biodiversity, supporting pollinators and other wildlife with their consistent blooms [2]. Their adaptability to a range of climates and soil conditions further extends their utility, ensuring that they can thrive in various

environments [3]. As a result, extensive research is performed in order to continuously improve the existing propagation and production methods of perennials.

Micropropagation, a cutting-edge technique in horticulture, offers numerous advantages for the efficient and rapid propagation of plants. This method involves the in vitro cultivation of plant tissues, enabling the production of large quantities of disease-free and genetically identical plantlets [4]. Micropropagation has revolutionized the horticultural industry by significantly reducing the time required for plant multiplication, ensuring the preservation of elite plant germplasm, and enabling year-round production [5]. Nonetheless, despite the obvious benefits of micropropagation, the technique also faces some challenges, for example, during rooting and acclimatization, which require consideration. Another bottleneck is post-acclimatization plant management, since to maintain high quality, acclimatized plants require instant transfer to their place of destination—the final plant producer.

Inducing rooting remains a critical bottleneck [6]. Efficient root formation is indispensable for the successful acclimatization and establishment of in vitro-derived plants in the soil. Root development is influenced by various factors, including genotype-specific responses, growth medium composition (especially the presence of auxins), and physical parameters in the growth room [7].

Acclimatization is another critical challenge in micropropagation, during which the transition of in vitro-grown plantlets to the ex vitro environment takes place. This crucial phase involves adapting plants to the unique environmental conditions they will encounter in the glasshouse or field [8]. Factors such as differences in temperature, humidity, and light parameters can pose significant hurdles to achieving successful acclimatization [9,10]. Innovative strategies and protocols to enhance the acclimatization process, ultimately ensuring the survival and robust growth of micropropagated plants, are needed.

Simultaneous rooting and acclimatization of in vitro-produced plantlets represent a promising and efficient approach to plant production [11]. This innovative technique offers several advantages, including a reduction in labor and time required for plantlet production, and therefore, lowered costs, as well as enhanced survival rates during the transfer to ex vitro conditions [12]. The approach is particularly effective if performed with the use of light-emitting diodes (LEDs). Unlike traditional lighting sources, LEDs are highly energy-efficient, emitting light in wavelengths tailored to the specific needs of plants [13]. Their ability to provide a precise spectrum, including red and blue light, can promote photosynthesis and control plant morphology, resulting in healthier and faster-growing crops [14].

Another important factor that may significantly contribute to the production of high-quality plant material is the application of various microbiological preparations. Biostimulants refer to substances derived from a living organism or its metabolites, other than fertilizers, that enhance the growth of plants when used in small amounts. They can be produced from a number of bioproducts from natural sources. This includes humic substances, plant extracts, and polysaccharides, e.g., chitosan. Biopreparations can also contain beneficial microorganisms, bacteria, or fungi [15]. The interaction between plants and microorganisms plays a pivotal role in the ecosystem and in sustainable crop production [16]. Soil microorganisms enhance nutrient acquisition and stress tolerance to pathogenic encounters that challenge plant health [17].

Recently, commercially available microbiological biostimulants containing beneficial bacteria and/or fungi have emerged as a useful tool in horticulture and agriculture, revolutionizing the approach to crop cultivation and soil management [18]. These preparations offer an eco-friendly alternative to traditional agricultural practices by promoting soil health, enhancing plant growth, and mitigating the use of synthetic chemicals [19]. Microbiological preparations encompass a diverse array of microorganisms, such as bacteria, fungi, and mycorrhizal fungi, which form mutualistic associations with plants [20]. These microorganisms can fix nitrogen, solubilize phosphates, and suppress plant pathogens, contributing to improved nutrient uptake, disease resistance, and overall plant vigor [21].

Additionally, microbiological preparations play a significant role in soil remediation and bioremediation by breaking down pollutants and toxins, thus aiding in environmental sustainability [22]. However, to date, little attention has been paid to the use of microbiological preparations in the production of ornamental perennials.

The aim of this study was to investigate the influence of commercial microbiological preparations on the quality of in vitro-derived plants of popular ornamental perennials stored for six months after simultaneous rooting and acclimatization in a semi-sterile growth room under controlled conditions. The results of our research can be implemented in the large-scale production of ornamental plants.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

In vitro-derived, unrooted cuttings of the following perennial plant species were used in this study: *Brunnera macrophylla* 'Silver Heart', *Echinacea purpurea* 'Secret Glow', *Heuchera* × *hybrida* 'Northern Exposure Red', *Persicaria amplecicaulis* 'JS Caliente', and *Rudbeckia* × *hybrida* 'Sunbeckia Sophia Yellow' (Figure 1).

Figure 1. Unrooted in vitro-derived cuttings of five selected perennials prepared for planting and acclimatization: (**A**) *Brunnera macrophylla* 'Silver Heart'; (**B**) *Echinacea purpurea* 'Secret Glow'; (**C**) *Heuchera* × *hybrida* 'Northern Exposure Red'; (**D**) *Persicaria amplecicaulis* 'JS Caliente'; and (**E**) *Rudbeckia* × *hybrida* 'Sunbeckia Sophia Yellow'.

Sixty in vitro-derived cuttings from each cultivar per treatment were planted in multi-pot trays (single cell of 10 mL volume, 264 cells per tray) filled with SoMi peat substrate

(HAWITA Gruppe GmbH, Vechta, Germany) and sprayed with indole-3-butyric acid (IBA) auxin solution (1.5 mg·L^{-1}) to induce rhizogenesis. Simultaneous rooting and acclimatization were performed for four weeks in a semi-sterile growth hall with controlled climate parameters at Vitroflora Grupa Producentów Spółka z o.o. in Trzęsacz, Poland (Figure 2). Plants were grown on shelves equipped with LED lightbars (Spectrolight, Łódź, Poland) emitting red and blue light (R:B 50:50) with a photon flux density (PPFD) of 50 µmol·m^{-2}·s^{-1} and 12/12-h day/night photoperiod. The air temperature was 23 °C, and the relative air humidity was approximately 95%.

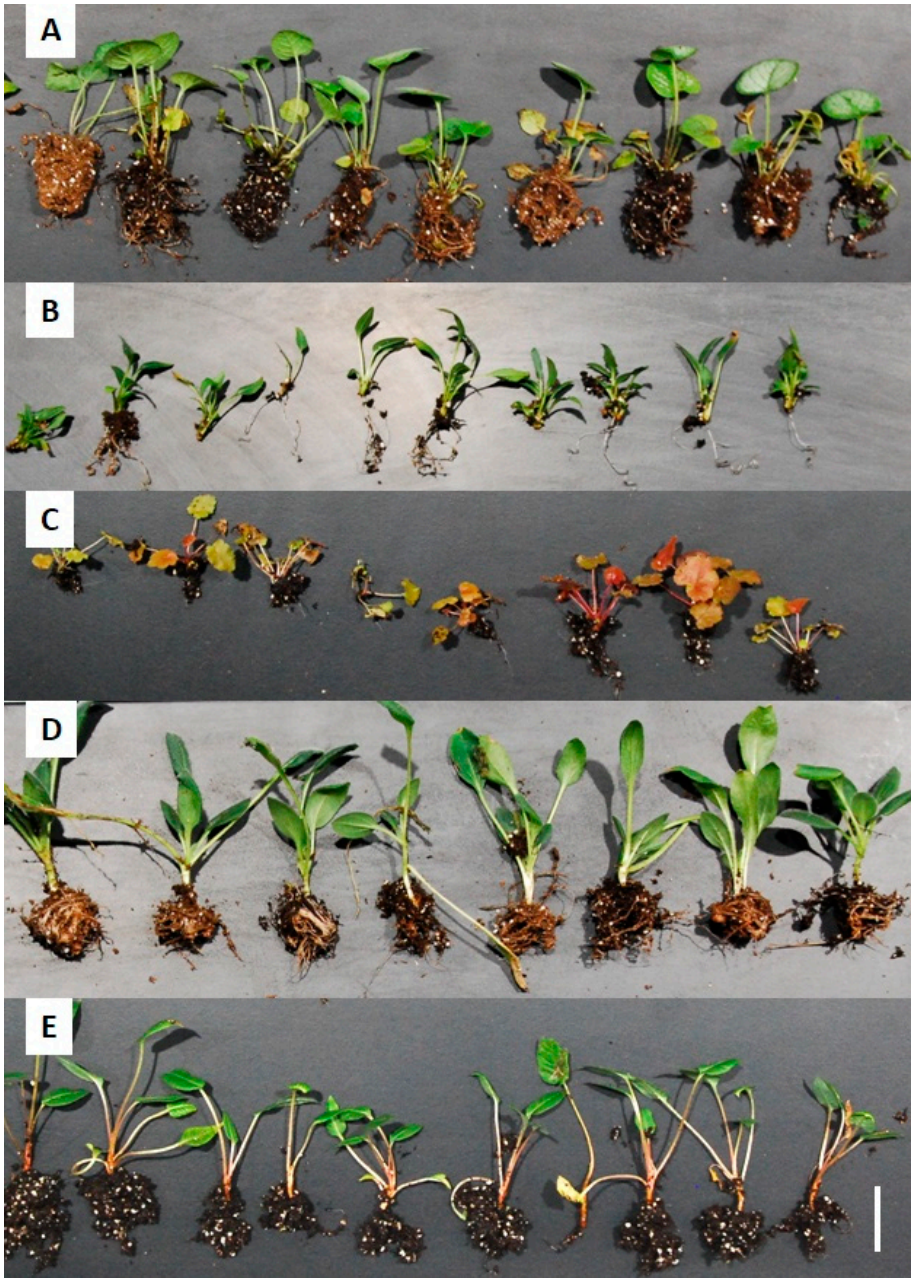

Figure 2. Plants of five selected perennials after four weeks of rooting and acclimatization, prior to biostimulant treatment: (**A**) *Brunnera macrophylla* 'Silver Heart'; (**B**) *Echinacea purpurea* 'Secret Glow'; (**C**) *Heuchera* × *hybrida* 'Northern Exposure Red'; (**D**) *Persicaria amplecicaulis* 'JS Caliente'; and (**E**) *Rudbeckia* × *hybrida* 'Sunbeckia Sophia Yellow'. Bar = 3 cm.

Next, after rooting and acclimatization were complete, the air temperature was lowered to 20 °C, and the humidity was set at 70%. The plants were cultivated in a semi-sterile

growth hall with controlled climate parameters for six months under LED lamps with a 30% red:70% blue light ratio, a PPFD value of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and a 12-h photoperiod. They were fertilized weekly with a 0.002% solution of commercial fertilizer (Peters Professional, ICL Polska, Warsaw, Poland, N:P:K 20:10:20). During this long-term storage, the plants were treated with selected microbiological preparations. The share of living plants (survival) was indicated monthly for each cultivar and treatment.

Finally, after six months of storage, the plants were transplanted into 3.5 cm-diameter paper-pot plugs (filled with SoMi peat substrate), placed in multi-trays, and transferred to a greenhouse, where they were cultivated at 17–19 °C ambient temperature, natural light conditions, and 60–70% air humidity for another three weeks for further biometrical analyses.

2.2. Microbiological Preparations Used in This Study

Three commercial biostimulants were used in this study. These microbial preparations were applied monthly (for six months) in the form of foliar sprays at concentrations recommended by the producers. The preparations were:

A. BactoFungiStop (BactoTech, Toruń, Poland), which contains a mixture of live soil bacteria (plant growth-promoting rhizobacteria of the *Bacillus* genus) collected and selected from natural habitats and probiotic microorganisms that restore the microbiological balance of the soil, support the decomposition of organic substances, stimulate the biosynthesis of phytohormones, vitamins, and amino acids, colonize plant tissue, and induce plant systemic responses, as recommended by the manufacturer for preventing fungal infections; concentration: 1.0% (v/v).
B. AzotoPower (BioLider, Łódź, Poland), which contains a high concentration of isolates of bacteria from the *Azotobacter* and *Arthrobacter* genera, supports the fixation of atmospheric nitrogen and makes it available to plants, thereby stimulating plant growth, enhancing the biosynthesis of phytohormones, improving the development of root systems and the efficacy of plant nutrition, and supporting plant stress resistance; concentration: 0.05% (w/v).
C. Guard (Target, Kartoszyno, Poland), which contains, according to the manufacturer's description, "useful strains of bacteria" that restore the microflora of above-ground parts of plants, protect the plants against the development of diseases, and accelerate the regeneration of plants after stress of various origins; concentration: 0.4% (w/v).

A non-treated control was also included.

2.3. Biometrical and Statistical Analyses

Biometric measurements included the shoot length, the number of leaves per plant, and a detailed analysis of the leaf and root system architecture. The leaf architecture, which includes measurements of the total leaf area, perimeter, length, maximum width, and average width, was assessed using a Perfection V800 Photo scanner (EPSON, Suwa, Japan), as well as the WinFOLIA™ 2016b and XLFolia 2016a software (Regent Instruments, Québec City, QC, Canada). Based on those data, the aspect ratio (width to length ratio) and the form coefficient (a value that grades the leaf shape as between circular and filliform) were counted. Moreover, the chlorophyll content index (CCI) in leaves was measured in triplicate in each plant using a CCM 200 Plus chlorophyll meter (OPTI SCIENCES, Hudson, NH, USA). The root systems were cut off from the shoots and then scanned using the Perfection V800 photo scanner. The scanning was performed within transparent polypropylene cuvettes filled with water. Next, the images of the root systems were processed and analyzed using the WinRHIZO™ imaging software from Regent Instruments. This analysis included measurements of the total length of the roots, a mean root system area, volume, and root diameter, as well as the number of forks (branches) and tips [13]. Five randomly selected plants (replications) from each experimental object were included in the analyses.

The experimental data were presented as mean ± standard error (SE). The results were evaluated using the one-factor analysis of variance (ANOVA) and the Duncan's Multiple Range Test at $p = 0.05$ using Statistica 12.0 (Tibco Software, Palo Alto, CA, USA).

3. Results

Spraying the leaves of *Brunnera macrophylla* with Guard microbiological preparation provided the highest survival rate (approx. 100%) that was constant throughout the experiment (Figure 3). On the other hand, in *Echinacea purpurea* and *Persicaria amplecicaulis*, a gradual decrease in plant survival was observed in the following months of storage, regardless of the biostimulant treatment (BactoFungiStop even had a deleterious effect on the plants' viability in *Persicaria amplecicaulis* compared to the other treatments and control). The survival of *Heuchera* × *hybrida* and *Rudbeckia* × *hybrida* was lower in plants sprayed with Azotopower and Guard than in the control and BactoFungiStop treatments (Figure 3).

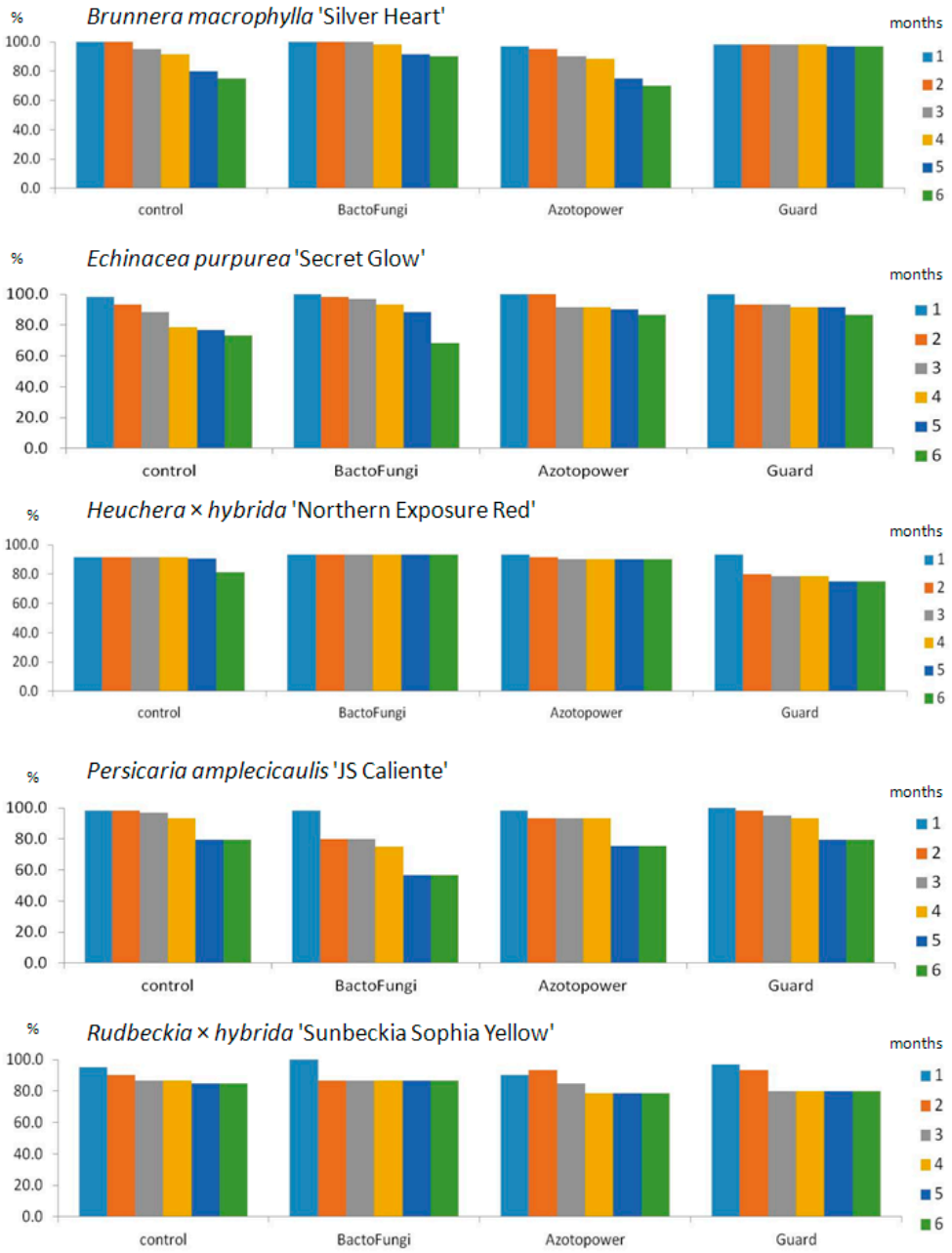

Figure 3. The share of living plants (%) of five perennials used in this study after each month of storage in the semi-sterile growth hall under controlled conditions as affected by microbial biostimulant application.

All of the studied microbiological preparations stimulated the elongation of shoots in *Brunnera macrophylla* 'Silver Heart'. No effect of the preparations on the number and development of leaves was found, except for the leaves treated with BactoFungiStop

and Guard, which were more filiform in shape (Figure 4). On the other hand, the tested preparations stimulated the development of the root system in terms of the most studied traits, i.e., root length, area, and volume, as well as the number of root forks and tips, compared to the control (Table 1).

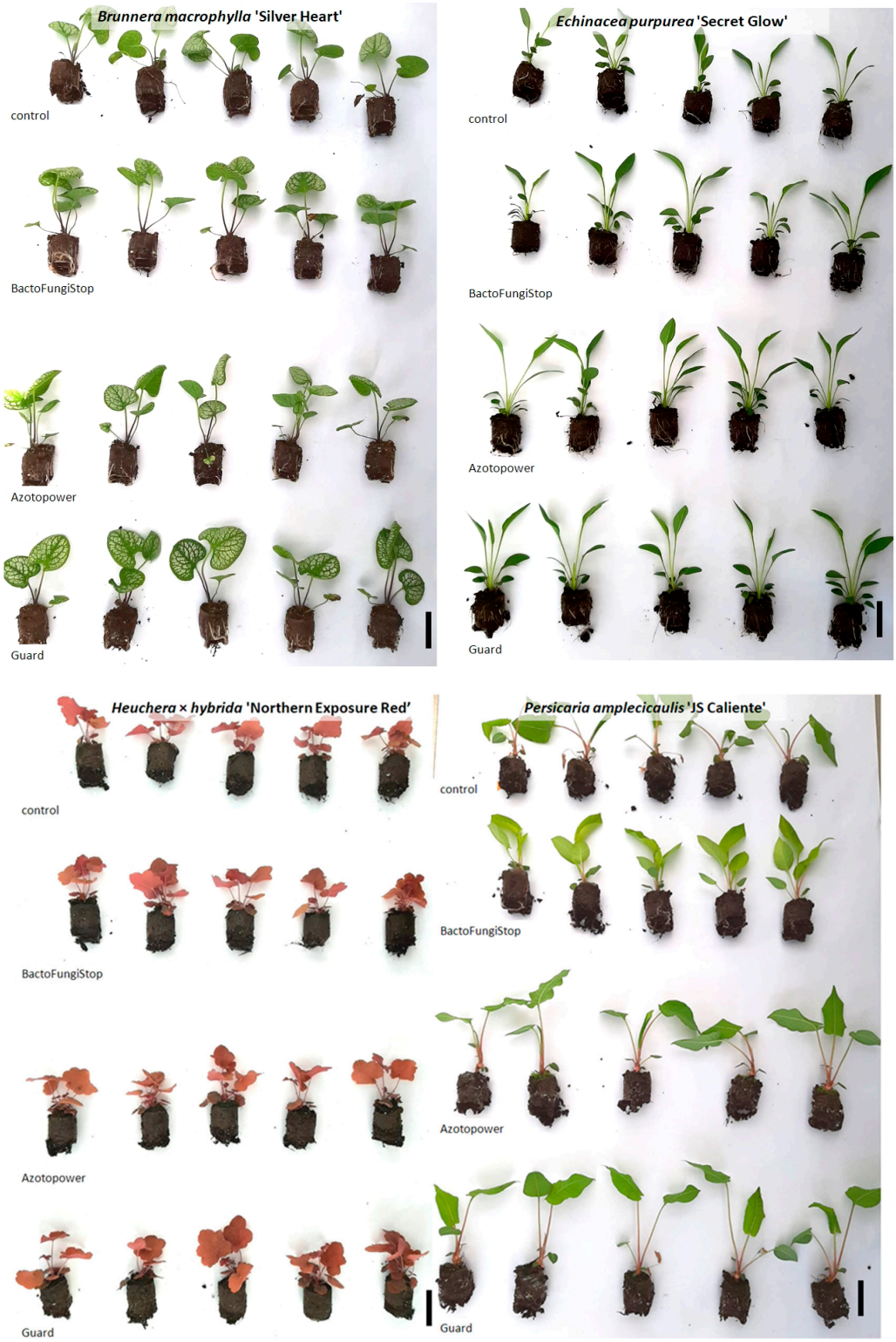

Figure 4. *Cont.*

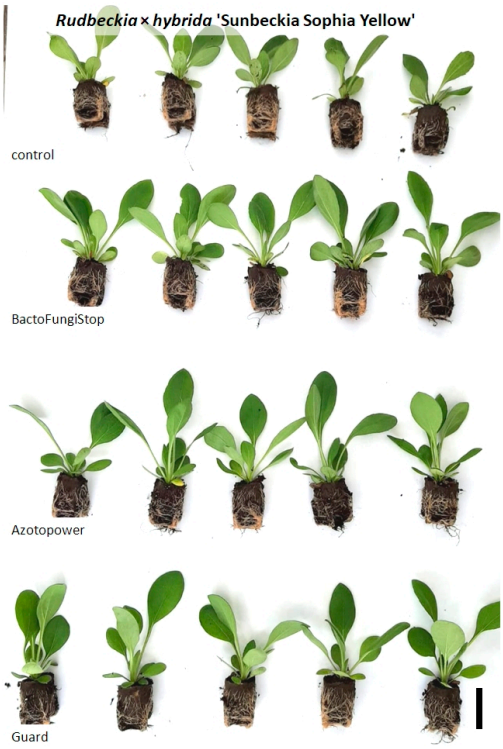

Figure 4. Plants of *Brunnera macrophylla* 'Silver Heart', *Echinacea purpurea* 'Secret Glow', *Heuchera* × *hybrida* 'Northern Exposure Red', *Persicaria amplecicaulis* 'JS Caliente', and *Rudbeckia* × *hybrida* 'Sunbeckia Sophia Yellow' after six-month storage in a semi-sterile growth hall under controlled conditions with monthly foliar application of microbiological preparations, followed by three weeks of growth in a greenhouse. Bar = 5 cm.

Table 1. Effect of microbiological preparation on the biometrical parameters of *Brunnera macrophylla* 'Silver Heart'.

Trait	Control	BactoFungiStop	AzotoPower	Guard
Shoot length (cm)	11.1 ± 0.19 b	12.6 ± 0.16 a	12.6 ± 0.19 a	12.5 ± 0.78 a
No. of leaves	4.6 ± 0.60 ab	3.6 ± 0.51 b	5.4 ± 0.40 a	3.8 ± 0.20 b
Chlorophyll content (CCI)	14.2 ± 0.42 a	15.0 ± 1.38 a	15.8 ± 0.81 a	16.6 ± 1.22 a
Leaf area (cm^2)	19.4 ± 0.62 a	22.2 ± 2.31 a	20.2 ± 2.02 a	22.7 ± 0.62 a
Leaf max. width (cm)	5.3 ± 0.06 a	5.7 ± 0.34 a	5.3 ± 0.31 a	5.7 ± 0.13 a
Leaf avg. width (cm)	2.0 ± 0.09 a	2.1 ± 0.19 a	1.9 ± 0.19 a	2.2 ± 0.12 a
Leaf length (cm)	10.2 ± 0.24 a	11.4 ± 0.26 a	11.3 ± 0.16 a	11.1 ± 0.58 a
Leaf perimeter (cm)	30.4 ± 0.38 a	33.8 ± 0.84 a	32.5 ± 0.91 a	33.9 ± 1.28 a
Aspect ratio	0.51 ± 0.01 a	0.50 ± 0,02 a	0.47 ± 0.02 a	0.52 ± 0.03 a
Form coefficient	20.4 ± 0.38 b	33.8 ± 0.84 a	32.5 ± 0.91 ab	33.9 ± 1.28 a
Total root length (cm)	233.4 ± 6.13 b	357.0 ± 12.6 a	341.9 ± 21.3 a	373.2 ± 37.2 a
Root system area (cm^2)	45.0 ± 3.09 b	65.8 ± 3.20 a	71.2 ± 4.55 a	69.5 ± 6.74 a
Root diameter (mm)	0.613 ± 0.04 ab	0.586 ± 0.01 b	0.663 ± 0.01 a	0.593 ± 0.01 b
Root system volume (cm^3)	0.70 ± 0.09 b	0.97 ± 0.06 a	1.18 ± 0.08 a	1.03 ± 0.10 a
No. of forks in roots	1141 ± 54 b	1558 ± 80 a	1512 ± 144 ab	1846 ± 180 a
No. of root tips	336 ± 42 c	1463 ± 180 a	938 ± 115 b	1152 ± 197 ab

Means in rows marked with the same letters do not differ significantly at the significance level of $p = 0.05$, according to Duncan's test.

All three microbiological preparations enhanced the shoot length, leaf perimeter, root area, and root volume in Echinacea purpurea 'Secret Glow'. BactoFungiStop and Guard enhanced additionally the total root length and number of root tips. Among the studied preparations, Guard was the most effective. This preparation stimulated the production of

longer leaves compared to all other experimental objects, although there was no difference in leaf width (Figure 4). It also increased the number of root forks compared to the control (Table 2).

Table 2. Effect of microbiological preparation on the biometrical parameters of *Echinacea purpurea* 'Secret Glow'.

Trait	Control	BactoFungiStop	AzotoPower	Guard
Shoot length (cm)	11.9 ± 0.51 c	13.8 ± 0.54 b	14.8 ± 0.45 ab	15.9 ± 0.27 a
No. of leaves	9.8 ± 0.58 a	9.8 ± 0.37 a	10.4 ± 0.51 a	10.6 ± 0.51 a
Chlorophyll content (CCI)	16.3 ± 0.95 a	17.2 ± 0.83 a	16.8 ± 0.84 a	15.6 ± 0.49 a
Leaf area (cm^2)	6.6 ± 0.41 a	8.7 ± 0.98 a	8.5 ± 0.67 a	9.1 ± 0.79 a
Leaf max. width (cm)	1.9 ± 0.06 a	2.1 ± 0.14 a	2.0 ± 0.13 a	2.1 ± 0.16 a
Leaf avg. width (cm)	0.6 ± 0.03 a	0.7 ± 0.05 a	0.7 ± 0.05 a	0.7 ± 0.05 a
Leaf length (cm)	10.6 ± 0.60 b	12.6 ± 0.67 b	12.9 ± 0.18 b	13.5 ± 0.22 a
Leaf perimeter (cm)	23.6 ± 1.36 c	27.7 ± 1.42 b	29.5 ± 0.99 ab	32.0 ± 0.59 a
Aspect ratio	0.18 ± 0.01 a	0.17 ± 0.01 a	0.15 ± 0.01 a	0.15 ± 0.01 a
Form coefficient	23.6 ± 1.36 c	27.7 ± 1.42 b	29.5 ± 0.99 ab	32.0 ± 0.59 a
Total root length (cm)	94.4 ± 9.33 c	167.0 ± 18.9 ab	149.0 ± 16.5 bc	221.4 ± 31.2 a
Root system area (cm^2)	16.9 ± 1.44 c	28.9 ± 3.37 b	27.8 ± 3.09 b	40.4 ± 5.21 a
Root diameter (mm)	0.556 ± 0.01 a	0.550 ± 0.02 a	0.595 ± 0.01 a	0.580 ± 0.01 a
Root system volume (cm^3)	0.23 ± 0.02 c	0.40 ± 0.05 b	0.41 ± 0.05 b	0.58 ± 0.07 a
No. of forks in roots	351 ± 51 b	624 ± 74 ab	561 ± 78 ab	889 ± 178 a
No. of root tips	253 ± 33 b	778 ± 98 a	249 ± 30 b	740 ± 139 a

Means in rows marked with the same letters do not differ significantly at the significance level of $p = 0.05$, according to Duncan's test.

Plants of *Heuchera* × *hybrida* 'Northern Exposure Red' sprayed with microbiological preparations had longer shoots and leaves (Figure 4), a higher leaf perimeter, root area, and root diameter compared to the non-treated control. No difference was found between the efficiency of the three studied preparations in terms of these traits. However, plants treated with Guard additionally had leaves of higher area and width compared to the control (Table 3).

Table 3. Effect of microbiological preparation on the biometrical parameters of *Heuchera* × *hybrida* 'Northern Exposure Red'.

Trait	Control	BactoFungiStop	AzotoPower	Guard
Shoot length (cm)	6.7 ± 0.32 b	8.2 ± 0.25 a	8.3 ± 0.30 a	8.5 ± 0.21 a
No. of leaves	10.8 ± 1.83 a	10.8 ± 2.13 a	14.4 ± 2.56 a	11.4 ± 1.43 a
Chlorophyll content (CCI)	4.7 ± 1.51 a	3.4 ± 0.23 a	4.3 ± 1.10 a	3.4 ± 0.30 a
Leaf area (cm^2)	11.3 ± 0.90 b	13.6 ± 1.04 ab	13.1 ± 1.32 ab	15.0 ± 0.49 a
Leaf max. width (cm)	3.6 ± 0.17 b	4.0 ± 0.14 ab	4.0 ± 0.21 ab	4.2 ± 0.09 a
Leaf avg. width (cm)	1.80 ± 0.12 a	1.86 ± 0.08 a	1.80 ± 0.11 a	2.1 ± 0.06 a
Leaf length (cm)	6.3 ± 0.22 b	7.4 ± 0.28 a	7.3 ± 0.37 a	7.4 ± 0.15 a
Leaf perimeter (cm)	19.1 ± 0.69 b	22.3 ± 0.84 a	22.6 ± 0.93 a	23.4 ± 0.64 a
Aspect ratio	0.57 ± 0.02 a	0.55 ± 0.01 a	0.54 ± 0.02 a	0.58 ± 0.02 a
Form coefficient	19.1 ± 0.69 b	22.3 ± 0.84 a	22.6 ± 0.93 a	23.4 ± 0.64 a
Total root length (cm)	116.8 ± 3.84 a	114.4 ± 13.9 a	121.3 ± 14.1 a	111.9 ± 9.57 a
Root system area (cm^2)	16.9 ± 0.58 b	18.5 ± 2.00 a	20.2 ± 2.30 a	19.5 ± 1.54 a
Root diameter (mm)	0.460 ± 0.02 b	0.518 ± 0.01 a	0.533 ± 0.02 a	0.558 ± 0.01 a
Root system volume (cm^3)	0.19 ± 0.01 a	0.24 ± 0.02 a	0.27 ± 0.03 a	0.27 ± 0.02 a
No. of forks in roots	619 ± 16 a	629 ± 75 a	647 ± 73 a	570 ± 61 a
No. of root tips	603 ± 91 a	432 ± 86 a	650 ± 110 a	464 ± 49 a

Means in rows marked with the same letters do not differ significantly at the significance level of $p = 0.05$, according to Duncan's test.

As for *Persicaria amplecicaulis* 'JS Caliente', the use of BactoFungiStop had a negative impact on most shoot and leaf parameters compared to the other experimental treatments (Figure 4). In contrast, this preparation stimulated the development of longer roots with a greater area and a higher number of root tips compared to the control. AzotoPower increased the root diameter, while Guard promoted the development of longer leaves in this species (Table 4).

Table 4. Effect of microbiological preparation on the biometrical parameters of *Persicaria amplecicaulis* 'JS Caliente'.

Trait	Control	BactoFungiStop	AzotoPower	Guard
Shoot length (cm)	14.3 ± 0.43 a	12.0 ± 0.56 b	15.4 ± 1.17 a	16.5 ± 0.74 a
No. of leaves	6.4 ± 0.40 ab	7.6 ± 0.29 a	5.4 ± 0.85 b	5.6 ± 0.20 b
Chlorophyll content (CCI)	6.7 ± 0.32 a	3.5 ± 0.18 b	6.7 ± 0.79 a	6.6 ± 0.25 a
Leaf area (cm^2)	21.6 ± 2.04 a	15.3 ± 0.44 b	25.4 ± 1.44 a	23.7 ± 1.22 a
Leaf max. width (cm)	4.5 ± 0.17 a	3.4 ± 0.02 b	4.6 ± 0.12 a	4.5 ± 0.13 a
Leaf avg. width (cm)	1.8 ± 0.13 a	1.4 ± 0.01 b	1.9 ± 0.06 a	1.7 ± 0.10 a
Leaf length (cm)	11.9 ± 0.53 b	10.8 ± 0.32 c	13.4 ± 0.58 ab	14.0 ± 0.67 a
Leaf perimeter (cm)	32.6 ± 1.45 a	25.9 ± 0.61 b	33.6 ± 1.13 a	35.3 ± 1.61 a
Aspect ratio	0.38 ± 0.01 a	0.31 ± 0.01 b	0.34 ± 0.01 ab	0.33 ± 0.02 b
Form coefficient	32.6 ± 1.45 a	25.9 ± 0.61 b	33.65 ± 1.13 a	35.32 ± 1.61 a
Total root length (cm)	396.8 ± 43.2 b	572.1 ± 59.9 a	363.3 ± 40.2 b	293.6 ± 28.4 b
Root system area (cm^2)	35.1 ± 3.53 b	50.0 ± 6.02 a	35.3 ± 4.21 b	27.6 ± 3.10 b
Root diameter (mm)	0.283 ± 0.01 b	0.276 ± 0.01 b	0.309 ± 0.01 a	0.298 ± 0.01 b
Root system volume (cm^3)	0.25 ± 0.02 ab	0.35 ± 0.05 a	0.27 ± 0.04 ab	0.21 ± 0.03 b
No. of forks in roots	4172 ± 510 ab	5143 ± 585 a	3522 ± 361 b	2824 ± 256 b
No. of root tips	2145 ± 183 b	5629 ± 829 a	2967 ± 442 b	1966 ± 235 b

Means in rows marked with the same letters do not differ significantly at the significance level of $p = 0.05$, according to Duncan's test.

The used microbiological preparations affected positively the shoot length, number of leaves, leaf area, length, and perimeter in *Rudbeckia* × *hybrida* 'Sunbeckia Sophia Yellow' compared to the control; however, no significant differences between the actions of these preparations were found (Figure 4). None of the preparations stimulated the development of roots in rudbeckia. Contrarily, Guard had a negative impact on the root length, area, and volume (Table 5). Interestingly, none of the preparations affected the biosynthesis of chlorophyll in any of the studied species.

Table 5. Effect of microbiological preparation on the biometrical parameters of *Rudbeckia* × *hybrida* 'Sunbeckia Sophia Yellow'.

Trait	Control	BactoFungiStop	AzotoPower	Guard
Shoot length (cm)	9.7 ± 0.37 b	12.5 ± 0.57 a	12.7 ± 0.53 a	12.8 ± 0.28 a
No. of leaves	7.4 ± 0.40 b	11.0 ± 0.55 a	10.6 ± 0.75 a	8.2 ± 0.58 b
Chlorophyll content (CCI)	12.3 ± 1.27 a	11.1 ± 0.57 a	10.4 ± 0.56 a	10.8 ± 0.88 a
Leaf area (cm^2)	14.5 ± 1.26 b	19.1 ± 0.74 a	18.9 ± 1.42 a	19.2 ± 1.28 a
Leaf max. width (cm)	3.2 ± 0.22 a	3.7 ± 0.07 a	3.2 ± 0.28 a	3.7 ± 0.19 a
Leaf avg. width (cm)	1.6 ± 0.11 a	1.7 ± 0.06 a	1.5 ± 0.06 a	1.58 ± 0.07 a
Leaf length (cm)	9.3 ± 0.36 b	11.5 ± 0.44 a	11.0 ± 1.13 ab	12.1 ± 0.32 a
Leaf perimeter (cm)	22.1 ± 0.81 b	27.0 ± 0.90 a	25.5 ± 2.58 ab	28.2 ± 0.81 a
Aspect ratio	0.35 ± 0.02 a	0.32 ± 0.02 ab	0.28 ± 0.01 b	0.30 ± 0.01 ab
Form coefficient	22.1 ± 0.81 b	27.0 ± 0.90 a	25.5 ± 2.58 ab	28.3 ± 0.81 a
Total root length (cm)	731.6 ± 72.8 a	696.0 ± 75.9 ab	717.3 ± 54.8 a	505.2 ± 62.2 b
Root system area (cm^2)	128.8 ± 10.1 a	124.3 ± 8.79 a	121.0 ± 10.4 a	90.9 ± 9.16 b
Root diameter (mm)	0.566 ± 0.02 a	0.581 ± 0.04 a	0.536 ± 0.01 a	0.580 ± 0.02 a
Root system volume (cm^3)	1.81 ± 0.14 a	1.79 ± 0.14 a	1.63 ± 0.16 ab	1.31 ± 0.11 b
No. of forks in roots	3885 ± 419 a	3616 ± 596 a	3573 ± 294 a	2560 ± 380 a
No. of root tips	2481 ± 800 a	2994 ± 500 a	3480 ± 470 a	1861 ± 585 a

Means in rows marked with the same letters do not differ significantly at the significance level of $p = 0.05$, according to Duncan's test.

4. Discussion

With increasing global concerns about sustainable agriculture and horticulture, microbiological preparations are expected to play a pivotal role in addressing these challenges, offering a promising chance for sustainable and environmentally responsible practices [23]. This study investigated the influence of microbiological preparations on the quality of popular ornamental perennials stored for six months in a semi-sterile growth hall. The investigation revealed species-specific responses and differential effects on various growth parameters. For example, BactoFungiStop and Guard stimulated shoot elongation in *Brunnera macrophylla*, while AzotoPower and Guard were most effective in enhancing shoot length and leaf parameters in *Echinacea purpurea*. Likewise, *Brunnera macrophylla*, *Echinacea purpurea*, and *Heuchera* × *hybrida* exhibited increased root development in response to all the microbiological treatments compared to the non-treated control. On the other hand, *Persicaria amplecicauli* did not respond positively to most treatments (except for BactoFungiStop), while the rooting of *Rudbeckia* × *hybrida* was even reduced by Guard preparation. It is quite a common phenomenon that various plant species may react differently to the same microbiota [24,25]. The differential responses of the tested species to microbiological treatments likely result from variations in the specific physiological and genetic characteristics of each plant species, as well as the unique mechanisms of action associated with the applied microbiological preparations [26], as reviewed by Prisa et al. [27]. For example, rhizobacteria produce substances that affect the entire microbial community in the rhizosphere. They are capable of supplying nutrients, such as nitrogen, phosphorus, potassium, and essential minerals, or producing plant hormones [28]. The specific bacterial strains in BactoFungiStop, AzotoPower, and Guard may have different affinities for certain plant species, influencing the effectiveness of the treatments. The strains of *Azotobacter* and *Arthrobacter* genera in AzotoPower, for example, are involved in supporting nitrogen fixation, which may have different effects on plants with varying nitrogen requirements [29]. This could explain the positive responses found in *Brunnera macrophylla*, *Echinacea purpurea*, and *Heuchera* × *hybrida*, which may have benefited from increased nitrogen availability. BactoFungiStop and Guard, on the other hand, are designed to enhance disease resistance and mitigate stress. Therefore, the positive effects observed in the studied perennials may be linked to the plants' ability to develop proactive strategies to manage disease or cope with stress more effectively [30]. Microroganisms establish symbiotic relationships with plants, promoting nutrient exchange and triggering systemic responses. Several studies indicate that the presence of rhizobacteria and mycorrhizal organisms contributes to the promotion of plant growth, stimulation of phytohormone and siderophore production, phosphate solubilization, reduction in ethylene levels, and upregulation of genes associated with dehydration responses and antioxidant mechanisms [31]. Moreover, biocontrol agents can directly attack or compete with pathogens, preventing their establishment or reducing their population [32]. Further research may be needed to understand the underlying mechanisms and optimize the use of microbiological preparations for specific ornamental plants. The differential effects of the preparations on leaf length, width, and form coefficient indicate their potential role in shaping plant architecture [33]. As for *Rudbeckia* × *hybrida*, it is recommended to screen for other rooting stimulators as the microbiological preparations used here were ineffective. Different biostimulants contain various active ingredients, and not all may be suitable for rudbeckia or may address the specific needs of its root development. Perhaps the exogenous application of auxins in the form of foliage spray could be more effective in promoting root development, as reported in other plant species [34].

It was observed that none of the tested preparations significantly influenced chlorophyll biosynthesis in any of the studied species. Chlorophyll biosynthesis is a complex biochemical process regulated by various genetic, environmental, and physiological factors [35]. The microbiological preparations used in this study, designed to prevent fungal infections, support nitrogen fixation, and protect against diseases, may not have direct interactions with the chlorophyll biosynthetic pathways. Moreover, chlorophyll production is influenced by environmental factors such as light intensity, temperature, and nutrient

availability [36–38], and the controlled ambient conditions in the growth room have not allowed for significant variations in these parameters.

5. Conclusions

The use of commercial microbiological preparations as biostimulants significantly affected the growth and development of in vitro-derived plants of popular ornamental perennials after six months of storage. The microbiological preparations stimulated shoot elongation and root development in most studied perennials, with Guard demonstrating notable effectiveness across multiple traits in *Echinacea purpurea*, *Heuchera* × *hybrida*, and *Rudbeckia* × *hybrida*. However, negative impacts were also observed, such as Bacto-FungiStop's adverse effects on the shoot and leaf parameters in *Persicaria amplecicaulis* and Guard's negative influence on root characteristics in *Rudbeckia* × *hybrida*. Future research could explore the long-term effects of commercial microbiological preparations on the growth, flowering, and overall performance of ornamental perennials in field conditions.

Author Contributions: Conceptualization, N.M., A.T., A.W. and T.M.; methodology, N.M., A.T. and A.W.; software, D.K., N.M. and A.T.; validation, D.K., N.M., and A.T.; formal analysis, D.K., N.M., A.T. and A.W.; investigation, N.M., A.T., A.W., T.M. and J.W.; resources, T.M., D.K., N.M., A.T. and A.W.; data curation, D.K., N.M., A.T., A.W. and J.W.; writing—original draft preparation, D.K.; writing—review and editing, D.K., A.T. and N.M.; visualization, D.K. and N.M.; supervision, T.M., D.K. and N.M.; project administration, N.M., A.T., A.W. and T.M.; All authors have read and agreed to the published version of the manuscript.

Funding: This research received external funding from the National Centre for Research and Development, "Fast Track" competition, Smart Growth Operational Program 2014–2020, Measure 1.1 "R&D projects of enterprises", Sub-measure 1.1.1 "Industrial research and development work carried out by enterprises", project title "Development of an innovative technology for long-term ex vitro storage of cuttings in controlled climate conditions and spectrally optimized radiation of LED sources in a greenhousetron" (project no POIR.01.01.01-00-0467/2019), applicant Vitroflora Grupa Producers Sp. z o. o., Trzęsacz 25, 86-022 Dobrcz, Poland.

Data Availability Statement: Data are available by email on reasonable request.

Conflicts of Interest: The author Tomasz Michalik was employed by the company VITROFLORA Grupa Producentów Spółka z o.o. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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