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The Effect of Foliar Application of an Amino Acid-Based Biostimulant on Lawn Functional Value

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Abstract: The purpose of this research was to assess the functional value of the "Super Trawnik" lawn mixture. The studies were carried out between 2017 and 2019 at the Experimental Station of the University of Agriculture in Krakow (50°07′ N, 20°05′ E), and the experimental factor was the AGRO-SORB® Folium, a biostimulant containing amino acids and applied at three doses: 1, 2, and 3 L· ha^{-1}. Lawn visual quality was assessed on a 9-point scale, with 10–11 mowings at 4 cm during the growing period. An increase in the concentration of the stimulant applied as a spray resulted in a significant increase in its effectiveness; plants in plots with the highest dose of amino acid solution (Variant III) had the highest aesthetic and functional values. The AGRO-SORB® Folium reduced the occurrence of fungal diseases; compared to control plants, there was a 16% reduction of Fusarium patch (*Microdochium nivale*) infection and a 20% reduction of Dreschlera leaf spot (*Drechslera siccans*). Satisfactory effects were also recorded on plots where the product was applied at a dose of 2 L·ha^{-1} (Variant II). Those plots had more favourably rated turf, with higher resistance of plants to Fusarium patch by 12% and to Dreschlera leaf spot by 20% compared to control.

Keywords: amino acid application; grass lawn; turf appearance; overwintering; the structure of the leaf; Fusarium patch (*Microdochium nivale*); Dreschlera leaf spot (*Drechslera siccans-Helminthosporium disease*)

1. Introduction

Lawns are an important part of green areas, mainly because of their aesthetic features and unique recreational qualities. Grasses intended for lawns should produce dense even turf with attractive appearance, growing slowly after subsequent mowings [1]. One of the most important characteristics in the evaluation of lawns is their overall aesthetic value [2]. The assessment of this value, together with the assessment of the growth rate, determines the classification of a grass variety or lineage as adequate for lawns. In addition, the assessment of overall visual quality is highly related to other assessed characteristics such as turf density, growth rate, and leaf fineness [3].

One of the many factors that significantly affects lawn appearance is fertilizer treatment. On the market of lawn fertilizers, there are new biological products supporting the growth and development of plants and based on natural substances like amino acids, plant extracts, plant hormones, or humus substances [4]. Those products have a beneficial effect on grass metabolism, stimulating vital processes, reducing pathogen levels, and minimizing the effects of adverse environmental conditions like drought, salinity, or temperature fluctuations [5,6]. Those extreme conditions plants are exposed to can cause strong abiotic stress, and therefore it is increasingly recommended to use plant growth regulators or biostimulants in addition to basic mineral fertilizers, fungicides, and herbicides [7,8]. Regulation (EU) 2019/1009 of the European Parliament and Council (EC) defines plant biostimulants as "EU fertilizing product able to stimulate plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: (1) nutrient use efficiency, (2) tolerance to abiotic stress, (3) quality traits, or (4) availability of confined nutrients in the soil or rhizosphere" [9]. Real benefits in this respect have been documented, and the use of biostimulants is rising. Their action is based on strengthening natural tolerance and resistance of plants under stress and on stimulating genetically determined potential, causing better growth and development in effect [10,11]. Thus, biostimulants can improve the efficiency of traditional fertilizers and can also become an alternative to synthetic chemicals used in plant protection [12]. According to the European Biostimulants Industry Council, biostimulants in Europe are used on more than four million hectares, while other sources determine the area of crops treated with them as more than six million hectares [11]. According to Beaudreau [13], the potential for the use of biostimulants, together with increasing knowledge of their mechanism of action, will increase even more in the future. In conclusion, amino acid based product are becoming increasingly popular because they enhance growth and quality of plants by improving their overall condition and mitigating the negative impact of stress factors such as mentioned above drought, salinity, temperature fluctuations, and pathogen infection [7,14–16]. Biostimulants have a positive effect on the metabolism of grass species, stimulating life processes and eliminating the effects of adverse environmental conditions and pathogens [4,5]. Humus substances of various sources are currently used in many lawn treatment products [17]. It has been found that they have positive effects on grass seed germination, density, root system, and plant biomass [5]. Daneshvar et al. [18] attribute high-quality characteristics of ryegrass turf to treatment with substances containing plant growth hormones. After the application of humic acids to selected grass species plants respond to drought stress with, among others, an increase in antioxidant content [19]

However, there is still a lack of studies explaining fully how plants respond to such biological substances and how they affect physiological processes [10].

The purpose of the studies was to assess the effect of amino acid solution foliar application on the functional value of lawn turf.

2. Materials and Methods

2.1. Experimental Field

The research was conducted between 2017 and 2019 at the Experimental Station of the University of Agriculture in Krakow (50°07′ N, 20°05′ E), Poland, on the soil belonging to the Haplic Phaeozems (Siltic) developed from loess. Its pH_{KCl} was 6.8, and the content of macronutrients and micronutrients in soil dry matter was as follows: total N–2.52 $g·kg^{-1}$, P–64.23 $g·kg^{-1}$; K–160.47 $g·kg^{-1}$, Mg–42.51 $mg·kg^{-1}$, Cu–3.1 $mg·kg^{-1}$, Mn–242 $mg·kg^{-1}$, and Zn–53 $mg·kg^{-1}$. The experiment was set up according to standard recommendations for lawns [1,2]. The functional value of the lawn mixture "Super Trawnik" was assessed in the research. The mixture consisted of perennial ryegrass var. Stadion 12%, perennial ryegrass var. Maki 30%, tall fescue var. Fawn 20%, red fescue var. Aniset 25%, and red fescue var. Reverent 13%. The lawn mixture was sown at a rate of 22.5 $g·m^{-2}$ on 10 m^2 plots on April 5, 2017. The following doses of mineral fertilizers were applied in the year of sowing: 65 kg $N·ha^{-1}$, 33 kg $P·ha^{-1}$, 124.5 kg $K·ha^{-1}$, with 190 kg $N·ha^{-1}$, 35.2 kg $P·ha^{-1}$, 124.5 kg $K·ha^{-1}$ used in the following growing seasons. Nitrogen was used in the form of 34% (N) ammonium nitrate, phosphorous as superphosphate (17.4% P), and potassium in the form of potassium salt (49.8% K).

The experimental factor was the amino acid AGRO-SORB® Folium, a biostimulant (AGRO-SORB Polskie Aminokwasy; http://agro-sorb.com) sprayed at three doses: 1, 2, and 3 L·ha^{-1}. Plants sprayed with water only (being also a solvent to the biostimulant) served as control. Spray solutions were prepared by dissolving appropriate amounts of the biostimulant in such quantities of water as to make spraying liquid with the volume of 300 dm^3 ha^{-1}.

The AGRO-SORB® Folium biostimulant is a growth stimulant with 18 biologically active free amino acids (L-alpha) obtained by enzymatic hydrolysis. In its composition it contains biologically active free amino acids with the weight percentage of at least 9.3%, a minimum of 100 g per 1000 mL. The biostimulant contains the following amino acids: aspartic acid 0.450%, serine 0.321%, glutamate acid 1.814%, glycine 2.743%, histidine 0.208%, arginine 0.131%, threonine 0.323%, alanine 0.524%, proline 0.347%, cysteine 0.435%, tyrosine 0.174%, waline 0.551%, methionine 0.349%, lysine 0.661%, 0.308%, leucine 0.180%, phenylalanine 0.218%, and tryptophan 0.05% (the analysis of amino acid composition by high-performance liquid chromatography (HPLC) was carried out in the accredited laboratory of the National Research Institute of Animal Production in Kraków-Balice).

The following elements are included in the composition of the biostimulant: B (0.02%), Mn (0.05%), Zn (0.07%), and N (2.1%). The biostimulant was applied to leaves, three times during the growing season, at the beginning of April, June, and August at the following doses: Variant 1-1 L·ha^{-1}, Variant II-2 L·ha^{-1}, and Variant III-3 L·ha^{-1}.

Each year an average of 10–11 mowings were carried out at a height of 4 cm when the plants reached a height of 8–10 cm. The number and height of mowings were consistent with the recommendations of The Research *Centre* for Cultivar Testing (COBORU) for "relax" lawn mixtures [20].

2.2. Weather Conditions

Weather conditions during the experiment were generally favourable for the growth and development of lawn grasses. Annual rainfall in the study period (2017–2019) amounted to 634.8, 809.6 and 728.6 mm, respectively (Table 1). The rainfall during the growing period (April-September) was 338.6 mm in 2017, 572.2 mm in 2018, and 533.6 mm in 2019. Mean annual temperatures during the 2017–2019 period reached 9.6, 9.3, and 10.6 °C, with 16.2, 15.6, and 16.6 °C between April and September, respectively (Table 1).

Table 1. Rainfall and mean air temperature between 2017 and 2919, compared to mean multiannual temperature (1990–2019), at the Experimental Station in Prusy, University of Agriculture in Kraków.

Month	Rainfall [mm]				Mean Temperature (°C)			
	2017	**2018**	**2019**	**1990–2019**	**2017**	**2018**	**2019**	**1990–2019**
January	21.2	9.6	44.2	31.9	−2.1	−4.9	−2.1	−1.8
February	80.6	22.4	12.8	24.7	3.9	0.2	3.1	−0.3
March	34.6	43.8	21.4	34.4	4.7	6.4	6.2	3.6
April	58.6	111	76.2	50.2	9.5	7.6	10.3	9.1
May	41.4	83.8	205	79.4	14.5	14	12.4	13.6
June	59.8	45.2	22.4	73.8	18.8	18.8	22.2	16.1
July	92.8	84.4	53.2	85.3	19.6	19.2	19.2	18.2
August	62.0	83.8	88.2	82.0	18.5	20.3	20.5	18.9
September	24.0	164	88.6	70.6	16.3	13.5	14.7	16.5
October	104.4	83.0	36.0	49.7	7.7	9.9	11.3	8.8
November	36.2	48.4	43.0	35.7	3.8	4.4	6.1	3.7
December	19.2	30.2	37.6	29.2	0.3	1.9	3.2	−0.4
	Total (April–September)				Mean (April–September)			
	338.6	572.2	533.6	441.3	16.2	15.6	16.6	15.4
	Total				Mean			
	634.8	809.6	728.6	646.9	9.6	9.3	10.6	8.8

2.3. Assessment of Lawns

The functional value of the lawns was assessed based on the following parameters: overall appearance, turf density, color, overwintering, and susceptibility to disease. Plant disease severity was assessed using keys and graphic scales. The results of the observations were determined on a nine-point scale, in which the individual digits indicated the intensity of the characteristic, with number 9 designating the best rating and number 1 the worst [20]. Numbers from 1 to 9 for plant disease correspond to the following assessment: 1—very high (disease killed all the plants), 2—very high to high, 3—high (most plants killed), 4—high to moderate), 5—moderate (numerous patches of dead grass), 6—moderate to low, 7—low (some plants affected by disease), 8—low to very low and 9—very low (no disease symptoms). The color is specified on a scale of 1–9, where: 1—yellow-green, 2—olive green, 3—bright green, 4—green-grey, 5—juicy green, 6—green, 7—grass green, 8—dirty green, and 9—emerald. This system in lawn grass assessment is analogous to that described in the National Turfgrass Evaluation Program (NTEP), used in the USA and Canada. The collected plant material was analyzed for its chemical composition. The content of mineral components, i.e., calcium, magnesium, potassium, and sodium, was determined by atomic absorption spectrometry with FAAS atomization (Varian AA240FS Varian Inc., Palo Alto, Santa Clara, CA, USA), according to PN-EN 15505:2009 standard, while iron, manganese, and zinc according to PN-EN 14084:2004 standard. The content of copper was determined with a validated method of atomic absorption spectrometry with electrothermal atomization, using ET-AAS graphite cuvette (Varian AA240Z Varian Inc., Palo Alto, USA), as per PN-EN 14084: 2004 standard. Nitrogen content was determined by the Kiejdahl method. Total phosphorus content was established based on UV-VIS spectrophotometry and staining with ammonium monovanadate (V) and ammonium heptomolybdate following the sample mineralization as described in PN–ISO 13730:1999 standard [21].

2.4. Statistical Analysis

The results were statistically processed using GenStat version 18. Firstly, the normality of the distributions of the studied traits were tested using Shapiro–Wilk's normality test. A three-way analysis of variance (ANOVA) was carried out to determine the effects of year, dose and growing season as well as all interactions on the variability of overall aspect and density. The effect of growing season for both traits (overall aspect and density) was strong significant ($p < 0.001$) so these two traits (overall aspect and density) observed in three seasons (spring, summer and autumn) were analyzed independently as six traits. A two-factor ANOVA was then performed to verify hypotheses about the absence of a significant effect of years and doses as well as year-by-dose interaction on the variability of the observed characteristics. The means values and standard deviations were calculated for all observed traits. Fisher's least significant differences (LSDs) were calculated for individual traits and on this basis, homogeneous groups of genotypes were determined. The relationship between the characteristics was determined by Pearson's linear correlation coefficient.

3. Results

Weather conditions in 2017–2019 were generally conducive to the growth and development of lawn grasses. High air temperatures were accompanied by relatively higher-than-average rainfall, with the exception of May in 2017 and 2018, June and July in 2019, and August and September in 2017.

The observed characteristics followed normal distribution. The results of variance analysis indicated a statistically significant effect of growing seasons on the values of overall and specific lawn characteristics, with no impact on the content of macronutrients and micronutrients. The doses of the amino acid biostimulant significantly affected all characteristics, except for Na, Ca, Fe, Zn, and Cu content, but interaction between growing seasons and doses was not statistically significant for any of them (Table 2).

Table 2. Mean squares from two-way analysis of variance for lawn grass characteristics.

Source of Variation	Year	Dose	Year × Dose	Residual
Degrees of freedom	2	3	6	24
Overall aspect (spring)	0.3014 ***	4.8931 ***	0.0007	0.0118
Overall aspect (summer)	0.2372 ***	7.4251 ***	0.0014	0.0097
Overall aspect (autumn)	0.3544 ***	3.2425 ***	0.0002	0.0200
Density (spring)	0.2824 ***	3.8090 ***	0.0006	0.0101
Density (summer)	0.2961 ***	2.3202 ***	0.0003	0.0101
Density (autumn)	0.3121 ***	2.4814 ***	0.0004	0.0151
Overwintering	0.2499 ***	1.1152 ***	0.0002	0.0076
Leaf colour in autumn	0.2643 ***	6.6494 ***	0.0015	0.0193
Leaf structure (fineness)	0.2058 ***	2.0559 ***	0.0003	0.0133
Susceptibility to diseases (*Microdochium nivale*)	0.3365 ***	2.4829 ***	0.0004	0.0033
Susceptibility to diseases (*Drechslera siccans*)	0.2523 ***	4.7170 ***	0.0027	0.0022
N	0.1533 ***	0.2111 ***	0.0001	0.0054
P	0.0885 ***	0.1512 ***	0.0001	0.0070
K	1.1519	23.7768 ***	0.0564	0.4377
Na	0.0001	0.0014	0.0009	0.0007
Ca	0.1878	0.0992	0.0605	0.4466
Mg	0.0124	0.4557 ***	0.0015	0.0333
Mn	2.5580	43.722 ***	2.732	1.56
Fe	4.55	3.55	1.26	39.02
Zn	23.42	70.14	35.94	45.29
Cu	0.4175	0.4006	0.1835	0.5336

*** $p < 0.001$.

Amongst others, overall aspect ratings varied greatest in the summer (V = 12.93%), and, depending on the dose of amino acids, it ranged between 5.4 and 7.4 (Table 3). The amino acid biostimulant already in the first year of research significantly ($p \leq 0.05$) affected the ratings of the aesthetic value of the lawns. Plants on plots with higher doses of the stimulant (Variants II and III) were assigned higher scores ($p \leq 0.05$) than control plants.

Table 3. Assessment of overall lawn characteristics on a 9-point scale (mean values ± standard deviation).

Treatment ($L \cdot ha^{-1}$)	Year	Overall Aspect			Density			Overwintering
		Spring	Summer	Autumn	Spring	Summer	Autumn	
Control	2017	6.600 ± 0.100	5.400 ± 0.100	7.000 ± 0.100	6.500 ± 0.100	6.900 ± 0.100	7.167 ± 0.153	6.567 ± 0.058
	2018	6.501 ± 0.099	5.238 ± 0.074	6.845 ± 0.073	6.402 ± 0.099	6.796 ± 0.099	7.059 ± 0.151	6.468 ± 0.057
	2019	6.775 ± 0.103	5.543 ± 0.103	7.186 ± 0.103	6.672 ± 0.103	7.083 ± 0.103	7.357 ± 0.157	6.741 ± 0.059
	2017–2019	6.625d ± 0.15	5.394d ± 0.155	7.010d ± 0.168	6.525d ± 0.147	6.926d ± 0.153	7.194d ± 0.186	6.592c ± 0.130
Variant I: 1 $L \cdot ha^{-1}$	2017	7.400 ± 0.100	5.700 ± 0.100	7.400 ± 0.100	7.100 ± 0.100	7.300 ± 0.100	7.400 ± 0.100	6.700 ± 0.100
	2018	7.289 ± 0.099	5.615 ± 0.099	7.266 ± 0.067	6.993 ± 0.099	7.191 ± 0.099	7.289 ± 0.099	6.599 ± 0.099
	2019	7.596 ± 0.103	5.851 ± 0.103	7.596 ± 0.103	7.288 ± 0.103	7.493 ± 0.103	7.596 ± 0.103	6.878 ± 0.103
	2017–2019	7.428c ± 0.16	5.722c ± 0.135	7.421c ± 0.164	7.127c ± 0.156	7.328c ± 0.159	7.428c ± 0.160	6.726b ± 0.150
Variant II: 2 $L \cdot ha^{-1}$	2017	7.833 ± 0.058	6.500 ± 0.100	7.967 ± 0.208	7.600 ± 0.100	7.600 ± 0.100	7.767 ± 0.058	6.833 ± 0.058
	2018	7.716 ± 0.057	6.402 ± 0.099	7.827 ± 0.172	7.486 ± 0.099	7.486 ± 0.099	7.650 ± 0.057	6.731 ± 0.057
	2019	8.041 ± 0.059	6.672 ± 0.103	8.178 ± 0.214	7.801 ± 0.103	7.801 ± 0.103	7.972 ± 0.059	7.014 ± 0.059
	2017–2019	7.863b ± 0.15	6.525b ± 0.147	7.991b ± 0.230	7.629b ± 0.163	7.629b ± 0.163	7.796b ± 0.150	6.860b ± 0.134

Table 3. *Cont.*

Treatment ($L \cdot ha^{-1}$)	Year	Overall Aspect			Density			Overwintering
		Spring	Summer	Autumn	Spring	Summer	Autumn	
Variant III: 3 $L \cdot ha^{-1}$	2017	8.333 ± 0.153	7.400 ± 0.100	8.333 ± 0.153	8.000 ± 0.100	8.100 ± 0.100	8.367 ± 0.153	7.367 ± 0.115
	2018	8.208 ± 0.151	7.289 ± 0.099	8.208 ± 0.151	7.880 ± 0.099	7.978 ± 0.099	8.241 ± 0.151	7.256 ± 0.114
	2019	8.554 ± 0.157	7.596 ± 0.103	8.554 ± 0.157	8.212 ± 0.103	8.315 ± 0.103	8.588 ± 0.157	7.562 ± 0.119
	2017–2019	8.365a ± 0.20	7.428a ± 0.160	8.365a ± 0.202	8.031a ± 0.170	8.131a ± 0.171	8.399a ± 0.202	7.395a ± 0.168
$LSD_{0.05}$		0.16	0.14	0.19	0.15	0.16	0.17	0.14
Standard deviation		0.667	0.811	0.559	0.591	0.472	0.491	0.339
Variation coefficient		8.81%	12.93%	7.26%	8.07%	6.29%	6.37%	4.92%

a, b, c, d—means in columns marked with different letters differ significantly ($p \leq 0.05$).

Another assessed feature was turf density, that is, the number of leaf blades covering a unit area during the growing season. The more leaf blades covered the soil, the higher the rating was. In the experiment, turf density ratings ranged from 6.5 to 8.4, with the smallest on the control plot and on the one treated with 1 $L \cdot ha^{-1}$ amino acid solution (Variant I). Variants II and III were assigned significantly higher ($p \leq 0.05$) ratings than control plants. The overwintering ratings ranged from 6.6 to 7.4 and were determined by comparing numbers of live leaf blades before the winter, at the end of the growing period, and in the spring, one week after it started. Plants treated with higher doses of amino acids were assigned significantly higher ($p \leq 0.05$) ratings than control plants.

One of the specific characteristics determined in the experiment was leaf color. Grass treated with higher doses of amino acids was rated highest (Table 4).

Table 4. Assessment of specific lawn characteristics on a 9-point scale (mean values ± standard deviation).

Treatment ($L \cdot ha^{-1}$)	Year	Leaf Colour in Autumn	Leaf Structure (Fineness)	Susceptibility to Diseases	
				Fusarium Patch *Microdochium nivale*	Dreschlera Leaf Spot *Drechslera siccans*
Control	2017	5.967 ± 0.153	6.033 ± 0.208	7.633 ± 0.058	7.433 ± 0.058
	2018	5.817 ± 0.149	5.882 ± 0.203	7.442 ± 0.056	7.247 ± 0.056
	2019	6.055 ± 0.155	6.122 ± 0.211	7.746 ± 0.059	7.543 ± 0.059
	2017–2019	5.946d ± 0.168	6.013d ± 0.208	7.607d ± 0.142	7.408c ± 0.139
Variant I: 1 $L \cdot ha^{-1}$	2017	7.167 ± 0.153	6.333 ± 0.058	8.267 ± 0.058	8.167 ± 0.058
	2018	6.987 ± 0.149	6.175 ± 0.056	8.060 ± 0.056	7.963 ± 0.056
	2019	7.272 ± 0.155	6.427 ± 0.059	8.389 ± 0.059	8.287 ± 0.059
	2017–2019	7.142c ± 0.182	6.312c ± 0.121	8.238c ± 0.152	8.139b ± 0.151
Variant II: 2 $L \cdot ha^{-1}$	2017	7.600 ± 0.100	6.533 ± 0.058	8.567 ± 0.058	8.967 ± 0.058
	2018	7.410 ± 0.098	6.370 ± 0.056	8.352 ± 0.056	8.742 ± 0.056
	2019	7.728 ± 0.105	6.630 ± 0.059	8.693 ± 0.059	9.010 ± 0.018
	2017–2019	7.579b ± 0.164	6.511b ± 0.124	8.537b ± 0.157	8.907a ± 0.131
Variant III: 3 $L \cdot ha^{-1}$	2017	7.933 ± 0.153	7.167 ± 0.058	8.867 ± 0.058	9.000 ± 0.000
	2018	7.735 ± 0.149	6.987 ± 0.056	8.645 ± 0.056	8.775 ± 0.000
	2019	8.073 ± 0.129	7.272 ± 0.059	8.997 ± 0.059	9.000 ± 0.000
	2017–2019	7.914a ± 0.193	7.142a ± 0.134	8.836a ± 0.162	8.925a ± 0.113
$LSD_{0.05}$		0.17	0.15	0.15	0.13
Standard deviation		0.774	0.444	0.484	0.649
Variation coefficient		10.83%	6.836%	5.829%	7.773%

a, b, c, d—means in columns marked with different letters differ significantly ($p \leq 0.05$).

The stimulant also reduced the occurrence of plant fungal diseases. Compared to control, there was a 16% reduction of Fusarium patch infection and a 20% lower incidence of Dreschlera leaf spot on plots with the highest dose of amino acids. Satisfactory effects were also recorded on plots where the product was applied at a dose of 2.0 L·ha^{-1} (Variant II). Those plots had a higher, more favorably rated turf with 12% higher resistance of plants to Fusarium patch and 20% to Dreschlera leaf spot, compared to control plants.

The results presented in Table 5 indicate large differences between macronutrient content of the lawn grass mixture; the highest variability was recorded for Na (V = 31.21%) and the lowest for N (V = 4.27%). It was found that the application of the amino acid biostimulant at the highest dose resulted in a significant increase in the content of N, P, and Mg.

Table 5. The effect of the amino acid stimulant on macronutrient content (g·kg^{-1} DM) in plants of the lawn mixture (mean values + standard deviations).

Treatment (L·ha^{-1})	Year	Macronutrient Content (g·kg^{-1} DM)					
		N	P	K	Ca	Mg	Na
Control	2017	3.315 ± 0.038	2.503 ± 0.075	18.94 ± 1.276	4.372 ± 0.208	1.284 ± 0.147	0.065 ± 0.014
	2018	3.523 ± 0.040	2.660 ± 0.080	19.04 ± 1.023	4.986 ± 0.808	1.396 ± 0.244	0.083 ± 0.026
	2019	3.474 ± 0.040	2.623 ± 0.079	18.70 ± 1.261	4.802 ± 0.778	1.345 ± 0.235	0.083 ± 0.046
	2017–2019	3.53b ± 0.541	2.66b ± 0.542	18.9d ± 1.043	4.720a ± 0.633	1.342b ± 0.191	0.077b ± 0.029
Variant I: 1 L·ha^{-1}	2017	3.344 ± 0.063	2.597 ± 0.096	20.58 ± 0.208	4.467 ± 0.799	1.344 ± 0.105	0.068 ± 0.030
	2018	3.553 ± 0.067	2.760 ± 0.101	21.10 ± 0.213	4.582 ± 0.819	1.379 ± 0.107	0.098 ± 0.028
	2019	3.504 ± 0.066	2.721 ± 0.100	20.32 ± 0.205	4.412 ± 0.789	1.328 ± 0.104	0.095 ± 0.027
	2017–2019	3.57b ± 0.423	2.78b ± 0.532	20.67c ± 0.389	4.487a ± 0.699	1.350b ± 0.094	0.087ab ± 0.028
Variant II: 2 L·ha^{-1}	2017	3.513 ± 0.080	2.588 ± 0.090	22.60 ± 0.024	4.677 ± 0.782	1.310 ± 0.045	0.096 ± 0.027
	2018	3.733 ± 0.085	2.750 ± 0.095	23.18 ± 0.024	4.796 ± 0.802	1.344 ± 0.046	0.112 ± 0.016
	2019	3.681 ± 0.084	2.711 ± 0.094	22.32 ± 0.024	4.619 ± 0.772	1.294 ± 0.045	0.108 ± 0.016
	2017–2019	3.73b ± 0.623	2.76b ± 0.478	22.7a ± 0.379	4.697a ± 0.685	1.316b ± 0.045	0.105a ± 0.019
Variant III: 3 L·ha^{-1}	2017	3.629 ± 0.091	2.798 ± 0.058	21.72 ± 0.650	4.622 ± 0.301	1.777 ± 0.272	0.109 ± 0.016
	2018	3.857 ± 0.097	2.973 ± 0.061	21.95 ± 0.147	4.741 ± 0.309	1.823 ± 0.279	0.070 ± 0.031
	2019	3.803 ± 0.096	2.932 ± 0.060	21.46 ± 0.642	4.565 ± 0.297	1.755 ± 0.269	0.068 ± 0.020
	2017–2019	3.88a ± 0.528	2.96a ± 0.418	21.71b ± 0.509	4.643a ± 0.273	1.785a ± 0.239	0.082ab ± 0.028
$LSD_{0.05}$		0.172	0.152	0.615	0.575	0.155	0.025
Standard deviation		0.157	0.126	1.554	0.580	0.251	0.027
Variation coefficient		4.267%	4.510%	7.401%	12.50%	17.31%	31.21%

a, b, c, d—means in columns marked with different letters differ significantly ($p \leq 0.05$).

Table 6 presents the weighted average content of micronutrients over the entire study period (2017–2019).The content of chemical elements in plant species varied, depending on the treatment; fluctuations were observed in the range of (mg·kg^{-1} DM): 1.58–2.04 Cu; 21.64–26.18 Mn; 11.14–12.50 Fe; 25.36–20.74 Zn. The highest variability in the content of micronutrients was recorded for Fe (V = 43.45%) and the smallest for Mn (V = 9.73%). When analyzing the micronutrient content of lawn mixtures treated with the amino acid biostimulant, it was found that the differences were insignificant, except for manganese and zinc.

Table 6. The effect of the amino acid stimulant on micronutrient content (g·kg^{-1} DM) in plants of the lawn mixture (mean values + standard deviations).

Treatment (L·ha^{-1})	Year	Micronutrient Content (mg·kg^{-1} DM)			
		Cu	Mn	Fe	Zn
Control	2017	1.491 ± 0.206	21.55 ± 1.644	10.29 ± 1.148	28.57 ± 12.649
	2018	1.591 ± 0.523	22.10 ± 1.686	11.71 ± 1.921	29.30 ± 12.972
	2019	1.669 ± 0.687	21.28 ± 1.624	11.43 ± 2.316	18.21 ± 9.437
	2017–2019	1.584a [1] ± 0.451	21.64b ± 1.475	11.14a ± 1.737	25.36a ± 1.539
Variant I: 1 L·ha^{-1}	2017	1.876 ± 1.071	22.45 ± 0.679	12.21 ± 3.686	18.61 ± 2.360
	2018	1.734 ± 0.713	23.02 ± 0.697	12.69 ± 4.026	19.09 ± 2.421
	2019	1.853 ± 1.058	22.17 ± 0.671	11.97 ± 3.525	18.38 ± 2.331
	2017–2019	1.821a ± 0.835	22.55b ± 0.701	12.29a ± 3.264	18.69b ± 2.077
Variant II: 2 L·ha^{-1}	2017	2.029 ± 0.782	24.70 ± 1.706	12.32 ± 10.251	19.89 ± 2.466
	2018	1.880 ± 0.787	25.33 ± 1.750	12.63 ± 10.513	20.40 ± 2.529
	2019	2.131 ± 0.741	26.39 ± 0.323	12.17 ± 10.124	23.59 ± 1.852
	2017–2019	2.014a ± 0.676	25.48a ± 1.438	12.37a ± 8.92	21.30ab ± 2.645
Variant III: 3 L·ha^{-1}	2017	1.690 ± 0.695	26.72 ± 0.327	10.89 ± 4.843	20.76 ± 5.485
	2018	1.765 ± 0.665	27.41 ± 0.335	13.56 ± 6.201	21.81 ± 7.576
	2019	2.663 ± 0.372	24.40 ± 1.685	13.06 ± 5.972	19.65 ± 2.436
	2017–2019	2.039a ± 0.697	26.18a ± 1.623	12.50a ± 5.09	20.74ab ± 4.922
$LSD_{0.05}$		0.652	1.303	5.241	6.236
Standard deviation		0.675	2.331	5.248	6.676
Variation coefficient		36.20%	9.727%	43.45%	31.02%

[1] a, b—means in columns marked with different letters differ significantly ($p \leq 0.05$).

Statistically significant positive correlation coefficients were found between all overall and specific lawn characteristics evaluated in the experiment (Figure 1). In addition, N, P, K, Mg and Mn content was positively correlated with all overall and specific lawn characteristics. Mn content was significantly correlated with Mg content ($r = 0.3338$), and so was Cu content with overall aspect rated in the autumn ($r = 0.3368$).

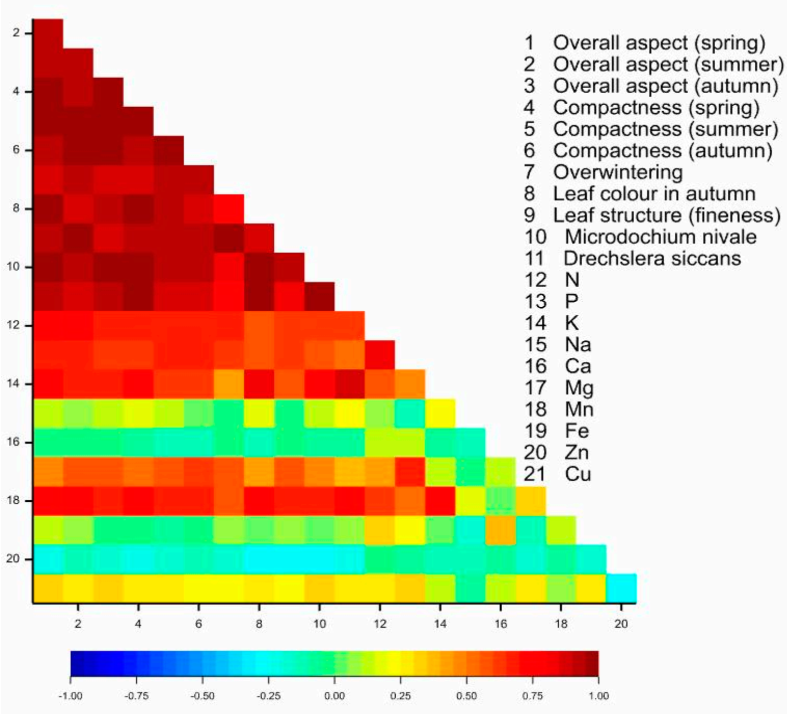

Figure 1. Heatmap for Pearson's linear correlation coefficients between observed traits ($r_{critical}$ = 0.32).

4. Discussion

The use of biostimulants in agriculture has been increasing in recent years, and research on the effects of these substances on lawn grasses has also been increasing. Studying the impact of commercial biostimulants on lawn turf quality, Mueller and Kussow [22] found a significant increase in the value of visual parameters such as colour and turf density. Zhang et al. [19] confirmed the positive effect of biostimulants in combination with basic mineral fertilizers on the visual quality of bentgrass turf. Butler and Hunter [23], in turn, recorded dark shade of green turf after the use of biostimulants. Those findings were confirmed by studies on perennial ryegrass (*Lolium perenne* L.) and *Kentucky bluegrass* (*Poa* pratensis L.) lawn mixtures [24], where the use of a biostimulant further reduced the rate of fungal diseases. Liu and Cooper [25] reported positive effects of biostimulants on photosynthesis and improvement of overall plant vigor. Van Dyke [26] presented a different view in research into biostimulant effects on the germination and initial growth of perennial ryegrass, with no significant differences between treated plants and control ones observed. Carey and Gunn [27] pointed out, however, that the positive impact of humus-based products on turf quality was evident in the adverse environment of plant growth with the occurrence of stress factors. Under favorable conditions, this effect might be masked and impossible to interpret unambiguously. Additionally, Carey and Gunn [27] observed significant impact of biostimulant treatments on the growth of fresh mass, improving grass vigour, with dark green turf. However, changes in color have not been confirmed in studies on chlorophyll content, as evidenced by the research of other authors [18,28].

In the present experiment the application of the amino acid based product to lawn turf at the highest dose resulted in a significant increase in the content of N, P, K, and Mg. Contrary to that, Van Dyke et al. [28] recorded decreased amounts of chemical elements in plant tissues. According to Carey and Gunn [27], the use of biological agents increases the level of potassium available to plants, which explains the increase in the resistance of grasses to pathogens. This effect is enhanced on clay soils, where the process of potassium leaching is limited. Nikbakht and Pessarakli [29] report about increased availability of phosphorus to plants treated with biostimulants, which can be explained by the fact that this chemical element forms complexes with iron. Hunter and Anders [30] stress a need for research into the application of biological substances to lawn grass, given the wide range of benefits declared by the biostimulant industry and the still few research reports providing reliable scientific data on this issue. Daneshvar et al. [18] recorded an increase in visual turf quality after biostimulant application, with increased iron content in plant tissues and a better formed root system of perennial ryegrass. Confronting the results of their research with the work of other authors, they concluded that the instability in the way plants reacted to the biostimulants was dependent on the species and biostimulant.

An additional benefit of the use of biostimulants, including amino acids, is the reduction of greenhouse gas emissions. It is very important nowadays because an increase in the concentration of greenhouse gases in the atmosphere results in climate warming. Application of amino acids to plants increases the efficiency of mineral treatment and decreases the amount of biomass produced (turf with a slight growth), lowering mowing frequency and thus reducing CO_2 emissions into the atmosphere. Reducing the use of chemical fertilizers and facilitates environmental protection [31,32], the implementation of natural products as plant growth stimulants does not disturb environmental homeostasis [33].

In the present experiment it was found that due to their multifaceted action at both biochemical and physiological levels, biostimulants can increase plant tolerance of stress factors. The results of the studies therefore confirm the effectiveness of biostimulants in increasing the efficiency of lawn care. Compared to control, biostimulant treatment resulted in an increase in the ratings of some overall and specific lawn characteristics. In particular, the results demonstrated a significant effect of the stimulant on such variables as turf density and susceptibility to disease. The treatment positively affected not only the appearance of plants (overall aspect, color, and leaf structure), but also the content of minerals, which can be associated with a number of direct and indirect interactive mechanisms, including stimulating enzymatic activity associated with carbon and nitrogen metabolism [34].

In conclusion, the improvement of lawn characteristics was due to the applied biostimulant. Plants treated with it had higher aesthetic and functional values, a higher resistance to fungal diseases, and increased content of minerals, both macro and micronutrients. Apart from containing more nutrients, they were in better condition. Additionally, an increase in the concentration of the biostimulant resulted in a statistically significant ($p \leq 0.05$) increase in its effectiveness. Finally, strong ($p < 0.001$) positive correlations were observed between overall aspect (spring, summer, and autumn), density (spring, summer, and autumn), overwintering, leaf color in autumn as well as leaf structure (Figure 1).

The biostimulant used in the experiment is recommended for foliar application to all types of crops, especially during stressful periods. Recommended for integrated crops, it improves the performance of plant protection products and reduces their use [16]. Apart from increasing overall visual quality of lawn turf, rich in nutrients and resistant to fungal diseases, amino acid stimulants as a supplementary fertilizer enhance grass growth and development. In view of their potential benefits, it would also be advisable to expand and update research on different plant groups with varying rates of exploitation.

5. Conclusions

Plants on plots treated with the highest dose of amino acids (Variant III) were assigned the highest aesthetic and functional rating. It was also found that the treatment reduced the occurrence of plant diseases; compared to control, there was a 16% reduction in Fusarium patch infection and a 20% reduction in Dreschlera leaf spot infection. Furthermore, increasing doses of amino acids resulted in a significant increase in N, K, Mg, Na, and Mn content in the mixture plants. On the basis of the results, it can be concluded that an increase in the concentration of the stimulant resulted in a significant increase in its effectiveness. It is advisable, therefore, to use amino acids at a higher dose (Variant III) to improve the aesthetic value of lawns.

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