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The Effect of Growth Activators and Plant Growth-Promoting Rhizobacteria (PGPR) on the Soil Properties, Root Yield, and Technological Quality of Sugar Beet

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Abstract: The strategy "from farm to fork" assumes a reduction in the usage of fertilizers and plant protection products in EU agriculture. The aim of this study, conducted over the years 2017–2019 in several locations in Poland, is to evaluate the application of growth activators with and without plant growth-promoting rhizobacteria to reduce mineral nitrogen fertilization without negative effects on the root yield. We studied the effect of these activators on selected soil properties. The experimental treatments included the application of the growth activators Penergetic (K + P) and Azoter, which contains the bacteria *Azotobacter chroococcum*, *Azospirillum brasilense*, and *Bacillus megaterium*, before sowing or during vegetation. The nitrogen rates were reduced by 30% in comparison to full nitrogen mineral fertilization (control treatment). In selected experiments, the application of Penergetic and Penergetic with Azoter caused a higher content of nitrate nitrogen ($N-NO_3$) and ammonium nitrogen ($N-NH_4$) after the sugar beet harvest as well as higher contents of mineral nitrogen (Nmin), P, K, and Mg in the soil in comparison to the treatment with the full dose of mineral nitrogen fertilization. The obtained results proved that it was possible to reduce the mineral application of nitrogen by 30% without a decrease in the biological and pure sugar yield, and even with an increase in the sugar yield caused by the application of the growth activators Penergetic (K + P) and Azoter.

Keywords: growth activator; nitrogen; plant growth-promoting rhizobacteria; sugar beet

1. Introduction

An expected 50% growth in the global population during the 21st century demands advanced technologies and low-input crop management, including precision delivery of nutrients at certain growth stages of crops [1].

In the crop production of sugar beet, new and effective methods of increasing sugar yields have been introduced. One of the innovations is the foliar application of silicon [2–9]. To protect the environment, efforts are typically made to limit the doses of mineral nitrogen. An innovative method is the application of the growth activators Penergetic P and K. A beneficial effect of the application of Penergetic-P for yield was proved in potatoes [10], sugar beet [11], snap-beans [12], and coffee [13]. A positive effect of Penergetic-K on grain yield and its quality was observed in organic wheat production [14] and soybeans [15]. A positive effect of the preparation on the seed germination energy and biometric traits of vegetables was also observed [16] as well for seedlings of winter wheat [17]. Nascente and Cobucci [18] observed positive effects of Penergetic-K and P application on the seed yield of common beans.

Various groups of bacteria that are able to stimulate plant growth by a mechanism(s) of action are referred to as plant growth-promoting rhizobacteria (PGPR). They affect plant growth and development directly or indirectly either by releasing plant growth regulators (PGRs) or other biologically active substances. This causes an increase in the content of available nutrients, which increase nutrient uptake and reduces the negative effects of pathogenic microorganisms on plants [19]. PGPRs have various types of effects, such as an increase in root growth and nutrient uptake, the stimulation of plant hormones, inhibition of the activity of plant pathogens, improvement of the soil structure, and mineralization of organic pollutants [20].

Biofertilizers increase nutrient availability and crop health without negative environmental effects [21]. Bacterial-based biofertilizers and the plant probiotics market is growing all around the world due to the need for sustainable crop production [22]. One example of the commercial application of *Azospirillum* sp. inoculants is the use in approximately 3.5 million ha in South America [23]. In the near future, plant growth-promoting bacteria (PGPB) will begin to replace the use of fertilizers and pesticides in agriculture [24]. Rhizospheric bacteria and fungi in the form of biofertilizers and biopesticides allow for reduced application of synthetic chemicals in crop production [25]. Plant growth-promoting rhizobacteria with probiotic potential can improve plant health and yield without negative environmental effects [26].

The aim of the study was to evaluate the effect of growth activators with and without plant growth-promoting rhizobacteria on sugar beet to avoid the negative effects of N fertilization on sugar yield and selected soil properties.

2. Materials and Methods

In the years 2017–2019, we conducted seven field experiments with sugar beet: three in 2017 (Sahryń, Pągów, and Stegienka), one in 2018 (Sahryń), and three in 2019 (Sahryń, Pągów, and Kępina) (Figure 1). The experiments were conducted on the following soils: Sahryń—Calcic Chernozem; and Pągów, Kępina, and Stegienka—Albic Podzols [27].

Figure 1. Location of the field experiments.

Soil samples were collected at two soil depths (0–30 and 30–60 cm) two times: immediately after harvesting the forecrop and after harvesting sugar beet. At the District Chemical and Agricultural Stations in Warszawa-Wesoła, Opole, and Gdańsk, the soil parameters were evaluated (pH_{KCl}) potentiometrically in 1 M KCl [28], for the content of soil organic carbon (SOC) [29], nitrate nitrogen (N-NO$_3$) and ammonium nitrogen (NH$_4$) [30], available phosphorus (P) [31], available potassium (K) [32], available

magnesium (Mg) [33], available boron (B) [34], available copper (Cu) [35], available iron (Fe) [36], available manganese (Mn) [37], and available zinc (Zn) [38].

　　The pH of the soil before the experiments was established as neutral or alkaline, except for Kępina in 2019 (in the layer from 0–30 cm) (Table 1). The pH of the SOC was in the range of 1.15–3.54 (0–30 cm) and 0.95%–2.97% (30–60 cm). For 0–30 and 30–60 cm, respectively, the $N\text{-}NO_3$ was at 5.7–81.5 and 6.6–23.4 mg kg^{-1}, the $N\text{-}NH_4$ was at 0.26–5.02 and 0.28–3.60 mg kg^{-1}, the mineral nitrogen (Nmin) was at 23–337 and 32–105 kg ha^{-1}, the P was at 46–145 and 27–155 mg kg^{-1}, the K was at 62–254 and 47–242 mg kg^{-1}, and the Mg was at 69–153 and 70–137 mg kg^{-1}. For 0–30 and 30–60 cm, respectively, the content of available micronutrients was (mg kg^{-1}): B—2.10–5.57 and 1.49–4.54, Cu—2.70–14.2 and 2.6–13.1; Fe—490–2573 and 520–2814, Mn—142–391 and 93–387, and Zn—5.9–26.4 and 4.8–25.8.

Table 1. Soil conditions before establishing the experiment with sugar beet (2016–2018).

Location	Soil Layer, cm	pH_{KCl}	SOC, %	mg kg^{-1}		Nmin, kg ha^{-1}	mg kg^{-1}							
				N-NO$_3$	N-NH$_4$		P	K	Mg	B	Cu	Fe	Mn	Zn
						2016								
Pągów	0–30	7.1	3.54	13.0	2.87	62	101.6	254	110	3.20	14.2	2333	391	26.4
	30–60	6.9	2.97	8.9	2.02	42	99.8	242	107	2.90	13.1	2526	387	25.8
Sahryń	0–30	7.3	2.11	81.5	5.02	337	46.2	104	71	2.80	6.4	540	164	6.3
	30–60	7.3	1.38	16.2	2.17	72	56.7	75	75	1.90	7.3	720	93	5.4
Stegienka	0–30	6.9	1.23	5.7	0.26	23	56.7	148	138	2.10	4.6	2573	142	10.7
	30–60	7.3	1.34	7.9	0.28	32	27.0	47	117	2.90	5.8	2814	198	14.1
						2017								
Sahryń	0–30	7.5	1.66	36.2	1.58	147	86.8	62	69	2.23	7.3	490	167	5.9
	30–60	7.8	1.02	6.6	3.60	40	5.7	21	70	1.49	5.7	520	110	4.8
						2018								
Kępina	0–30	7.5	1.15	25.3	<1.00	109	144.8	212	77	2.44	2.9	1601	156	6.5
	30–60	7.6	0.99	23.4	1.10	105	155.2	228	81	2.15	2.8	1564	151	5.9
Pągów	0–30	7.0	1.18	8.5	3.49	54	51.9	112	153	4.23	2.73	1546	163	14.6
	30–60	6.7	0.95	6.7	3.49	46	48.8	95	137	4.03	2.63	1523	158	14.3
Sahryń	0–30	7.3	2.76	18.4	3.11	93	90.7	133	99	5.57	8.8	630	157	8.0
	30–60	7.4	2.22	13.2	2.47	67	48.8	62	123	4.54	9.2	700	125	7.6

　　The amount of rainfall during the growing season (April–October) in the year 2017 was 443–615 mm, in 2018—466 mm, and in 2019, from 364 to 426 mm (Table 2). During the period of the highest demand of sugar beet for water (June–August), the optimal value of the k-factor was found only in 2017 in July and August 2019 in Pągów and in June 2019 in Kępina. Sugar beet was grown in Sahryń after winter rapeseed, and in other locations after winter wheat. The characteristics of the technology used in the experiments are presented in Table 3.

Table 2. Weather conditions during the growing season of sugar beet (2017–2019).

Location	Month	Precipitation, mm	Average Temperature, °C	Hydrotermical Coefficient, k
		2017		
Sahryń	April	36	8.6	1.40
	May	45	13.5	1.08
	June	34	18.2	0.62
	July	95	18.9	1.62
	August	12	20.8	0.19
	September	118	14.5	2.71
	October	103	10.1	3.29
	Sum	**443**	-	-

Table 2. *Cont.*

Location	Month	Precipitation, mm	Average Temperature, °C	Hydrotermical Coefficient, k
Stegienka	April	26	6.2	1.40
	May	23	12.4	0.60
	June	50	16.0	1.04
	July	109	16.8	2.09
	August	96	18.1	1.71
	September	144	14.0	4.50
	October	61	8.8	2.24
	Sum	**509**	-	-
		2018		
Sahryń	April	36	13.9	0.86
	May	47	17.0	0.89
	June	111	18.1	2.04
	July	181	20.4	2.86
	August	13	20.8	0.20
	September	39	16.1	0.81
	October	39	10.6	1.19
	Sum	**466**	-	
		2019		
Sahryń	April	19	9.5	0.67
	May	118	14.1	2.70
	June	59	21.8	0.90
	July	45	18.2	0.80
	August	137	19.9	2.22
	September	24	13.7	0.58
	October	24	11.4	0.68
	Sum	**426**	–	
Pagów	April	30	10.0	1.00
	May	97	12.0	2.61
	June	7	22.0	0.11
	July	35	17.0	0.66
	August	75	21.0	1.15
	September	80	14.0	1.90
	October	40	10.0	1.29
	Sum	**364**	-	-
Kępina	April	12	9.2	0.43
	May	37	12.3	0.97
	June	85	20.7	1.37
	July	64	17.6	1.17
	August	52	19.2	0.87
	September	68	14.2	1.60
	October	58	10.3	1.82
	Sum	**376**	-	-
Sahryń	April	40	9.3	1.43
	May	73	14.3	1.65
	June	73	17.7	1.37
	July	100	19.7	1.64
	August	61	18.9	1.04
	September	60	13.8	1.45
	October	51	8.4	1.96
	Sum	**457**	-	-

Table 2. *Cont.*

Location	Month	Precipitation, mm	Average Temperature, °C	Hydrotermical Coefficient, k
Average from multiyear *				
Location	Month	Precipitation, mm	Average Temperature, °C	Hydrotermical Coefficient, k
Pagów	April	37	9.5	1.30
	May	64	14.4	1.43
	June	64	17.9	1.19
	July	89	19.7	1.46
	August	57	19.0	0.97
	September	59	13.7	1.44
	October	49	9.0	1.76
	Sum	**418**	-	-
Stegienka/ Kępina	April	28	7.8	1.20
	May	49	12.9	1.23
	June	61	16.0	1.27
	July	87	18.5	1.52
	August	71	18.2	1.26
	September	58	13.8	1.40
	October	53	8.6	1.99
	Sum	**406**	-	-

* Precipitation: Sahryń (1991–2019), Pagów (1998–2019), Stegienka and Kępina (2001–2019). Temperature: Sahryń (2002–2019), Pagów, Stegienka and Kępina (2001–2019). Source: own study based on data from Institute IMGW-PIB, "Pagro" farm, Strzyżów Sugar Factory.

In the experiments during autumn, we applied (depending on location) Polifoska 6 fertilizer (6% N in ammonium form, 8.7% P as mono and diammonium phosphate, 24.9% K as potassium chloride, and 2.8% S as sulphate), potassium chloride (49.8% K as potassium chloride), and Korn-Kali—potassium chloride with the addition of magnesium salt (33.2% K, 3.6% Mg, 3% Na, and 5% S). In spring, before sowing, we applied Saletrzak Standard 27—ammonium nitrate with the addition of dolomite flour containing calcium and magnesium (13.5% N in the ammonium form and 13.5% N in the nitrate form, 1.4% Ca, and 2.4% Mg) or ammonium sulphate 32 (16% N in the ammonium form and 16% N in the nitrate form).

During the growing season in the experiment, at the six-leaf stage of sugar beet (Biologische Bundesanstalt, Bundessortenamt und Chemical Industry growth scale (BBCH 16)) and 14 days later, foliar nutrition was conducted with micronutrient fertilizers containing boron (2×300 g ha^{-1} B). Protection against weeds, diseases, and pests was performed in accordance with the recommendations of the Institute of Plant Protection—National Research Institute in Poznań.

In the experiment, three treatments were applied:

0— Control (full nitrogen fertilization dose depending on the location—from 112 to 175 kg ha^{-1} N);

1— Dose of mineral nitrogen lower by 30% in comparison to the full dose before sowing and during vegetation—from 78 to 123 kg ha^{-1} N; Penergetic-K (400 g ha^{-1}) on the straw of the forecrop before it was mixed with the soil; Penergetic-K (400 g ha^{-1}) with the first herbicide spray; Penergetic-P (300 g ha^{-1}) with a second herbicide spray in spring; and Penergetic-P (300 g ha^{-1}) at the six-leaf stage of sugar beet (Biologische Bundesanstalt, Bundessortenamt und Chemical Industry growth scale (BBCH 16));

2— Dose of mineral nitrogen lower by 30% in comparison to the full dose before sowing and during vegetation—from 78 to 123 kg ha^{-1} N; Penergetic-K (400 g ha^{-1}) + Azoter (10 dm^3 ha^{-1}) on the straw of the forecrop before it is mixed with the soil; Penergetic-K (400 g ha^{-1}) + Azoter (10 dm^3 ha^{-1}) in spring with the first herbicide spraying; Penergetic-P (300 g ha^{-1}) with the second herbicide spray; and Penergetic-P (300 g dm^3 ha^{-1}) at the six-leaf stage of sugar beet (BBCH 16).

Table 3. Characteristics of sugar beet production technology in the experiment (2017–2019).

Location	Forecrop	Straw Yield of Forecrop, t ha^{-1}	Cultivar of Sugar Beet	Mineral Fertlization, kg ha^{-1}	Sowing Date	Harvest Date	Length of Vegetation Period (Days)
				2017			
Sahryń	Rapeseed	4.00	Panorama KWS	N—160 (variant No 0) and 112 (variants No 1 and No 2); P—45.8; K—237; S—8.4	31st of March	12th of October	196
Pagów	Winter wheat	7.00	Artus	N—156 (variant No 0) and 109 (variants No 1 and No 2); P—39.7; K—302; S—13.0; Ca—61.5; Mg—37.2; Na—25.9	31st of March	17th of November	200
Stegienka	Winter wheat	6.00	Piast	N—119 (variant No 0) and 83 (variants No 1 and No 2); P—13.1; K—183; S—13.0; Ca—14.3; Mg—3.6; Na—3.0	7th of April	15th of September	130
				2018			
Sahryń	Winter rapeseed	6.90	Levanda KWS	N—175 (variant No 0) and 123 (variants No 1 and No 2); P—34.9; K—249; S—11.2; Ca—13.6; Mg—13.3	11th of April	7th of September	147
				2019			
Sahryń	Winter rapeseed	3.10	Toleranza KWS	N—159 (variant No 0) and 111 (variants No 1 and No 2); P—34.9; K—249; S—11.2; Ca—7.2; Mg—12	30th of March	26th of September	179
Pagów	Winter wheat	5.55	Tapir	N—140 (variant No 0) and 98 (variants No 1 and No 2); P—18.3; K—173; S—44.8; Mg—29.3; Na—15.1	23rd of March	12th of November	203
Kępina	Winter wheat	5.50	Levanda KWS	N—112 (variant No 0) and 78 (variants No 1 and No 2); P—15.7; K—174; S—23; Ca—43; Mg—23; Na—4.5	5th of April	15th of September	162

0—Control; 1—Penergetic (K + P); and 2—Penergetic (K + P) + Azoter.

Penergetic-K and Penergetic-P are growth activators, the compositions of which are reserved by the manufacturer. They are produced based on bentonite and molasses processed by a special technology. The product contains no other chemical substances. The Penergetic International AG Company produces Penergetic-P and -K, from bentonite clays subjected to the application of electric and magnetic fields. These products are used to increase the photosynthetic efficiency of plants (Penergetic-P) or to improve the performance of organic matter decomposing organisms of the soil (Penergetic-K).

Azoter is a preparation that contains plant growth-promoting rhizobacteria (PGPR). The total number of living microorganisms (*Azotobacter chroococcum*, *Azospirillum brasilense*, and *Bacillus megaterium*) is at least 4×10^9 KTJ cm^{-3}, pH = 5.8–8.5.

The number of replications was 4, and the total number of plots was 12. Each plot consisted of six rows. The dimensions of a single plot were a length of 16 m and width of 2.7 m (43.2 m^2), of which 21.6 m^2 (three middle rows) was used for harvesting. During harvest, the plants were topped by hand on the three middle rows, and the leaves were weighed. The roots were then counted, dug up, and weighed. During the harvest, each plot was collected in accordance with the Polish Standard [39]. The root samples were transported to the Plant Breeding Station of the Kutno Sugar Beet Breeding Company in Śmiłów, where they were processed into pulp. The pulp was transported to Straszków, where the technological quality of the roots was evaluated on the automatic Venema technological line [40]: the sugar content polarimetrically [41], the K and Na by photoelectric flame photometry [41], and the α-amino nitrogen by fluorometric methods [42].

The measurements performed in the experiments were as follows:

- Plant density at harvest (thousand plants ha^{-1});
- Root yield (t ha^{-1});
- Yield of leaves (t ha^{-1});
- Yield of fresh biomass (t ha^{-1}) as a sum of the root yield (t ha^{-1}) and yield of leaves (t ha^{-1});
- Harvest index (HI) as a ratio of root yield to fresh biomass;
- Foliage coefficient as a ratio of yield of leaves to root yield;
- Fresh biomass of root (kg) as a ratio of root yield (kg) and number of plants per plot at harvest;
- Fresh biomass of leaves per plant (kg) as a ratio of (kg) and number of plants per plot at harvest;
- Plant fresh weight (kg) as the sum of fresh root mass (kg) and leaves of a single plant (kg);
- Content of sucrose in roots (%);
- Content of α-amino nitrogen in the roots (mmol kg^{-1});
- Content of potassium (K) in the roots (mmol kg^{-1});
- Content of sodium (Na) in the roots (mmol kg^{-1});
- Biological yield of sugar (t ha^{-1}) = product of root yield (t ha^{-1}) and content of sugar in roots (%);
- Pure sugar yield (t ha^{-1}) = root yield (t ha^{-1}) × [content of sugar (%) − sugar yield losses (%)] [43];
- Sugar yield losses (%) = standard molasses losses (%) + 0.6 (%);
- Standard molasses losses (%) = 0.012 × (K + Na) + 0.024 (α-amino nitrogen) + 0.48; where the content of K, Na and α-amino nitrogen are given in mmol kg^{-1} of pulp;
- Refined sugar content (%) = sucrose content (%) − sugar yield losses (%);
- Sugar productivity (%) = refined sugar content (%)/sugar content (%) × 100;
- Alkalinity coefficient W_A = (content of K (mmol kg^{-1}) + content of Na (mmol kg^{-1}))/content of α-amino nitrogen (mmol kg^{-1}) [44].

The data were analyzed using analysis of variance and multiple comparison of means using Tukey's procedure. The significance level for all the analyses was set at 0.05. The analyses were performed using Statistica 13 software (TIBCO Software Inc., Palo Alto, CA, USA). Descriptive statistics, such as the minimum, maximum, standard deviation (SD), and coefficient of variation (CV) were calculated.

3. Results

In certain locations, we observed that combinations No 1 and 2 were characterized by a higher content of nitrate nitrogen (N-NO$_3$) and ammonium nitrogen (N-NH$_4$) and, consequently, higher amounts of mineral nitrogen (Nmin) and higher contents of P, K, and Mg in the soil in comparison to the control treatment, 0 (Table 4).

Table 4. Soil conditions after the harvest of sugar beet (2017–2019).

Location	Treat-Ment	Soil Layer, cm	pH$_{KCl}$	SOC, %	mg kg^{-1} N-NO$_3$	N-NH$_4$	Nmin, kg ha^{-1}	mg kg^{-1} P	K	Mg	B	Cu	Fe	Mn	Zn
							2017								
Pagów	0	0–30	7.5	1.17	9.7	0.67	44	220.2	305	104	2.37	4.6	1687	231	35.4
		30–60	7.6	0.80	5.5	0.69	27	82.8	258	107	1.73	3.6	1348	139	16.6
	1	0–30	7.1	1.08	16.7	0.60	74	180.9	385	125	1.98	3.1	1348	191	17.8
		30–60	7.0	0.63	19.5	0.55	86	93.3	244	104	1.38	2.2	1086	134	8.5
	2	0–30	6.8	0.92	9.2	0.81	43	97.7	191	118	1.57	2.6	1273	178	13.1
		30–60	6.7	0.43	4.9	1.15	26	75.4	102	110	0.92	1.9	1146	98	6.8
Sahryń	0	0–30	7.5	2.40	17.0	1.1	73	8.7	79	76	4.36	7.2	380	118	13.0
		30–60	7.7	1.44	11.8	3.17	56	5.2	29	66	2.69	5.6	350	121	10.2
	1	0–30	7.6	2.22	22.2	<1.00	86	14.4	50	58	3.90	6.2	310	118	14.9
		30–60	7.9	1.11	15.2	2.03	67	5.2	25	56	2.16	5.1	305	103	6.7
	2	0–30	7.4	3.54	41.5	1.56	168	24.9	58	67	4.76	7.3	550	176	13.5
		30–60	7.6	2.16	22.7	2.54	98	8.3	33	81	3.56	7.7	430	122	24.0
Stegienka	0	0–30	7.2	1.38	7.9	<1	34.6	88.5	195	120	2.61	6.2	3894	259	11.8
		30–60	7.3	1.19	9.3	<1	40.3	95.5	239	124	2.70	6.2	3527	279	11.3
	1	0–30	6.9	1.68	8.4	<1	36.5	98.5	144	100	2.61	5.7	3104	230	10.1
		30–60	7.2	1.22	8.6	<1	37.5	109.0	177	108	2.73	5.5	2444	199	9.7
	2	0–30	6.7	1.26	10.9	<1	46.4	147.4	317	131	2.55	5.2	2936	252	9.7
		30–60	6.7	1.47	7.3	<1	32.5	143.0	369	122	2.74	5.4	3042	246	10.0
							2018								
Sahryń	0	0–30	7.2	4.98	84.3	1.54	335	11.8	37	64	5.80	8.8	900	159	9.0
		30–60	7.4	2.88	74.5	1.26	295	7.4	21	78	4.08	7.9	630	112	7.5
	1	0–30	7.4	3.18	61.4	3.67	254	43.2	46	84	5.02	9.0	720	151	8.0
		30–60	7.4	1.80	19.2	1.45	81	7.0	25	94	2.94	7.3	395	65	5.7
	2	0–30	7.4	3.18	22.1	1.41	92	26.2	42	92	5.80	9.2	775	153	10.0
		30–60	7.4	2.70	18.8	1.80	80	5.7	21	89	3.11	8.5	485	56	6.1
							2019								
Kępina	0	0–30	6.0	0.84	13.3	<1.00	51.9	116.8	168	93	0.96	2.9	1659	106	6.1
		30–60	6.5	0.81	8.5	<1.00	33.1	141.3	162	61	0.99	2.8	1546	111	6.3
	1	0–30	5.9	1.21	14.7	<1.00	57.3	136.0	177	76	1.07	2.7	1604	138	6.0
		30–60	6.1	0.74	10.7	<1.00	41.7	137.8	220	73	1.07	2.7	1627	132	6.1
	2	0–30	7.3	0.93	13.8	<1.00	53.8	228.9	213	62	1.68	2.6	1630	134	6.5
		30–60	7.2	1.26	7.8	<1.00	30.4	222.4	242	69	1.13	2.7	1839	134	6.8
Pagów	0	0–30	6.5	1.50	8.2	1.67	42	61.0	208	96	1.62	2.7	947	123	11.3
		30–60	6.4	1.08	4.7	1.42	26	25.7	114	121	1.06	2.2	1000	103	6.8
	1	0–30	6.6	0.81	7.3	1.75	39	73.7	164	98	1.77	3.8	986	125	12.6
		30–60	6.5	1.42	5.3	1.82	31	34.4	103	96	1.18	2.8	869	87	6.0
	2	0–30	7.4	1.04	8.4	2.44	47	119.9	199	89	1.84	3.2	771	135	14.5
		30–60	7.6	0.94	4.4	1.86	27	51.9	112	79	0.87	3.1	567	87	5.8
Sahryń	0	0–30	7.4	2.82	47.4	<1.00	185	66.3	71	90	6.79	8.3	606	167	6.0
		30–60	7.4	2.46	60.7	1.43	242	6.1	21	116	4.31	9.2	431	78	7.3
	1	0–30	7.4	2.64	35.2	<1.00	137	76.3	58	70	5.10	7.8	582	155	5.9
		30–60	7.4	1.44	36.2	<1.00	141	13.1	25	89	2.93	7.5	678	90	4.0
	2	0–30	7.3	1.86	30.3	<1.00	118	31.4	50	69	4.58	7.0	695	158	5.6
		30–60	7.6	1.04	52.5	1.02	209	4.4	17	71	2.42	6.8	762	111	4.3

0—Control; 1—Penergetic (K + P); and 2—Penergetic (K + P) + Azoter.

Variant No 1 (by 5.5%) and variant No 2 (by 5.1%) had significantly greater plant density during harvest in relation to the control object (Table 5).

Table 5. The influence of Penergetic activators (Penergetic International AG, Romanshorn, Switzerland) and Azoter bacterial preparation (Azoter Trading, Bratislava, Slovakia) on the yield, the technological quality of the roots and traits of sugar beet plants (2017–2019), and the effects of treatment, environment (location × year), and their interaction.

Trait	Treatment			p-Value Based on ANOVA		
	0	1	2	Treatment (T)	Environment (E: Year × Location)	Inter-Action: T × E
Plant density at harvest, thousand plants ha^{-1}	90.16 a *	95.10 b	94.71 b	0.054	<0.001	0.735
Yield of leaves, t ha^{-1}	49.95 a	47.75 a	53.99 b	0.002	<0.001	<0.001
Yield of roots, t ha^{-1}	84.46 a	90.65 b	94.17 b	<0.001	<0.001	0.051
Yield of roots and leaves, t ha^{-1}	134.41 a	138.40 a	148.16 b	<0.001	<0.001	0.002
Biological yield of sugar, t ha^{-1}	14.20 a	15.05 b	16.00 c	<0.001	<0.001	0.003
Pure sugar yield, t ha^{-1}	12.41 a	13.19 b	14.07 c	<0.001	<0.001	0.002
Harvest Index	0.64 a	0.66 b	0.65 ab	0.052	<0.001	<0.001
Foliage coefficient	0.60 b	0.54 a	0.58 ab	0.027	<0.001	<0.001
Content of sucrose in roots, %	16.80 ab	16.63 a	16.96 b	0.109	<0.001	0.001
The content of α-amino nitrogen in the roots, mmol kg^{-1}	21.36 a	21.11 a	20.89 a	0.894	<0.001	0.176
Potassium content in the roots, mmol kg^{-1}	39.84 b	35.15 a	36.09 a	<0.001	<0.001	0.002
Sodium content in the roots, mmol kg^{-1}	3.53 b	3.22 ab	2.90 a	0.125	<0.001	0.019
Standard molasses losses, %	1.51 b	1.45 a	1.45 a	0.052	<0.001	0.396
Sugar yield losses, %	2.11 b	2.05 a	2.05 a	0.052	<0.001	0.396
Refined sugar content, %	14.69 ab	14.58 a	14.91 b	0.106	<0.001	0.001
Sugar productivity, %	87.29 a	87.57 ab	87.75 b	0.121	<0.001	0.188
Alkalinity coefficient	2.24 b	1.91 a	1.96 a	0.001	<0.001	<0.001
The fresh mass of the leaves of the plant, kg	0.55 b *	0.51 a	0.57 b	0.014	<0.001	<0.001
Fresh root mass, kg	0.94 a	0.96 a	1.00 a	0.136	<0.001	0.002
Fresh plant biomass, kg	1.49 ab	1.47 a	1.57 b	0.038	<0.001	0.002

* The same letters within rows indicate a lack of significant differences between means at α = 0.05.

The use of Penergetic activators (variant No 1) resulted in a significant increase in the root yield (by 7.3%), harvest index value (by 3.1%), biological sugar yield (by 6.0%), and pure sugar yield (by 6.3%) and a significant reduction in the potassium content in the roots (by 11.8%), the foliage coefficient (by 10.0%), the standard molasses losses (by 4.0%), the sugar yield losses (by 2.8%), and the value of the alkalinity coefficient (by 14.7%) compared to the control object (Table 5, Figure 2).

Combination No 2 demonstrated a significant increase in the yield of leaves (by 8.1%), the yield of roots (by 11.5%), the total yield of roots and leaves (by 10.2%), the biological yield of sugar (by 12.7%), the pure sugar yield (by 13.4%), and the sugar productivity (by 0.5%) and a significant reduction in the potassium (by 9.4%) and sodium (by 17.9%) content in roots, the standard molasses losses (by 4.0%), the sugar yield losses (by 2.8%), and the value of the alkalinity coefficient (by 12.5%) in relation to the control combination.

The Penergetic + Azoter facility was characterized by a significantly higher yield of leaves (by 13.1%), sugar content in the roots (by 2.0%), total yield of roots and leaves (by 7.1%), fresh mass of the plant leaves (by 11.8%), fresh mass of the plant (by 6.3%), biological yield of sugar (by 6.3%), pure sugar yield (by 6.7%), and content of the refined sugar (by 2.3%) compared to the Penergetic object.

For the majority of the studied traits, a significant effect of the environment (years × location) was observed as well as a significant interaction between the treatments and environments, which indicates that the effect of the combinations varied in different years and locations.

Among the examined traits, the highest variability was found in the sodium content in the roots (CV = 49.3%), and the lowest variability was found in the sugar productivity (CV = 2.3%) (Table 6).
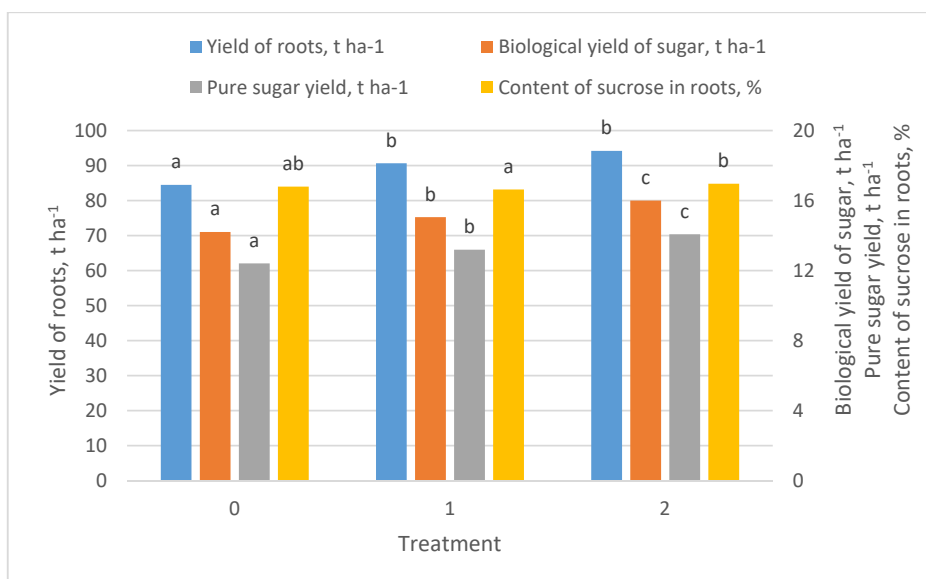
Figure 2. The influence of Penergetic activators and Azoter bacterial preparation on the yield of roots, sugar yield, and sucrose content (2017–2019). (The same letters under the columns in the same color indicate a lack of significant differences between means at $\alpha = 0.05$).

Table 6. Descriptive statistics for all experiments with sugar beet (2017–2019).

Trait	Mean	Minimum	Maximum	Standard Deviation (SD)	Coefficient of Variation (CV), %
Plant density at harvest, thousand plants ha^{-1}	93.32	65.00	112.04	11.89	12.74
Yield of leaves, t ha^{-1}	50.56	21.60	106.48	21.91	43.34
Yield of roots, t ha^{-1}	89.76	61.11	117.66	15.38	17.14
Yield of roots and leaves, t ha^{-1}	140.3.	93.06	204.63	28.51	20.31
Biological yield of sugar, t ha^{-1}	15.08	9.87	21.14	2.98	19.76
Pure sugar yield, t ha^{-1}	13.23	8.32	18.96	2.75	20.79
Harvest Index	0.65	0.45	0.81	0.09	13.81
Foliage coefficient	0.57	0.24	1.24	0.25	43.44
Content of sucrose in roots, %	16.80	14.45	20.29	1.55	9.22
The content of α-amino nitrogen in the roots, mmol kg^{-1}	21.12	11.00	44.10	6.86	32.46
Potassium content in the roots, mmol kg^{-1}	37.03	27.00	55.00	6.63	17.90
Sodium content in the roots, mmol kg^{-1}	3.22	1.15	13.40	1.59	49.29
Standard molasses losses, %	1.47	1.16	2.20	0.22	15.23
Sugar yield losses, %	2.07	1.76	2.80	0.22	10.82
Refined sugar content, %	14.73	12.31	18.28	1.64	11.15
Sugar productivity, %	87.53	81.45	90.30	2.03	2.32
Alkalinity coefficient	2.04	1.05	4.14	0.55	27.19
The fresh mass of the leaves of the plant, kg	0.54	0.22	1.10	0.22	40.14
Fresh root mass, kg	0.97	0.56	1.42	0.14	14.49
Fresh plant biomass, kg	1.51	0.85	2.11	0.26	17.24

4. Discussion

We obtained, in our study, the increase in the amount of mineral nitrogen in the soil after sugar beet harvesting in combinations with Penergetic activators. This confirmed the results of previous studies in which the use of the Penergetic-K (300 g ha^{-1}) activator in pre-sowing spraying in organic winter wheat cultivation resulted in an increase in pH, redox potential, and specific soil electric conductivity [14]. The process of mineralization increased when the oxidation was stronger, and this

caused an increase in the amount of mineral nitrogen in the soil after the crop harvest. The combined use of Penergetic with phosphorus and potassium mineral fertilization promoted the activity of fauna and microorganisms present in the 0–8 cm soil layer in soybeans. The activity of fauna and microorganisms in the 9–16 cm soil layer was intensified with the use of Penergetic, alone or with phosphate and potassium fertilization, in soybeans. The use of Penergetic did not significantly increase the soil biological activity in the winter crop [45].

The use of the activators Penergetic-K and Penergetic-P combined with a reduced dose of mineral nitrogen (reduced by 30%), increased the yield of the roots by 7.3%, the fresh root mass by 2.1%, the biological yield of sugar by 6.0%, and the pure sugar yield by 6.3% and reduced the sugar content of the roots by 0.17 p.p. in comparison with the control treatment (without the Penergetic activators and with a full dose of mineral nitrogen). In the literature, there are few results of experiments with the use of Penergetic activators in the cultivation of sugar beet. In one, foliar spray of Penergetic-P (0.1 dm^3 ha^{-1}) applied in the growth stage of BBCH 18–19 of sugar beet caused an increase in the before-harvest content of chlorophyll *a* (by 5.6%), chlorophyll *b* (by 8.6%), chlorophyll *a* + *b* (by 6.4%), and carotenoids (by 3.0%) in comparison to the control [11]. Root yield increased by 14.5%, and the average root mass increased by 9.1%. However, the sucrose content in the roots increased by 0.34 p.p., and the yield of white sugar increased by 17.2%.

The results of experiments with the use of Penergetic activators in the cultivation of crops other than sugar beet are available in the literature. The application of Penergetic-P in potato cultivation increased the share of large tubers from 39% to 55.9% and reduced the proportion of small tubers from 32.0% to 19.3% and, consequently, increased the yield of tubers by 16.6% in comparison to the control [10]. The application of Penergetic before sowing (dose 300 g ha^{-1}) in organic winter wheat caused an increase in grain yield by 5.0% in comparison to the control and an improvement of grain quality (increase in the protein content by 2.4% and gluten by 2.9%) [14].

Penergetic in soybeans did not affect the plant growth. However, the grain weight per plant and yield of soybeans were higher (by 20%) in comparison with the application of the fertilizer NPK and micronutrients [15].

Soil and foliar application of Penergetic-P in spring wheat caused an increase in the grain yield in comparison to wheat grown without such application. Penergetic-P applied to the soil and by foliar application resulted in a substantial increase in the number of seeds per spike, and lengthening of the stems compared with wheat in the control treatment [46].

In a greenhouse experiment performed in Brazil in green beans, the dry matter yield of commercial pods was not significantly different between objects with Penergetic-P and Penergetic-K and full mineral fertilization (NPK) versus objects with Penergetic-P and Penergetic-K with a 25% dose of NPK [12]. Penergetic-P positively influenced the germination of radish and tomato seeds as well as the growth and development of cucumber, red beet, tomato, and radish seedlings [16]. The use of Penergetic-P on the sowing day for winter wheat seeds in organic cultivation had a positive effect on the health of seeds and the root system assessed at the beginning of autumn during tilling (BBCH 21–23). Infestation of cocci by *Fusarium* spp. was reduced from 13.5% in the control to 9.0%, and root diseases were reduced from 71.7% to 55.0% [17].

There are also experimental results proving the beneficial effect of the Penergetic growth activators on the nutrition and yield of coffee, and the effect was dependent on the cover plants on the plantation [13]. Nascente and Cobucci [18] evaluated the effect of the application of Penergetic-K and Penergetic-P on the yield of common beans with diversified fertilization with phosphorus. A greater availability of P to plants when Penergetic was applied was possibly due to the soil colloids and organics or partly due to the increased microbial activity in the soil.

There are no results from studies with the use of Penergetic activators together with plant growth-promoting rhizobacteria. In our own research, we observed that the use of Penergetic activators together with the Azoter activator containing bacteria *Azotobacter chroococcum*, *Azospirillum brasilense*, and *Bacillus megaterium* caused an increase in the yield of roots by 3.9%, the fresh root mass by 4.2%,

biological yield of sugar by 6.3%, pure sugar yield by 6.7%, and sugar content in the roots by 0.33 p.p. in comparison to the object with only Penergetic activators.

Inoculation by *Azospirillum* sp. of dryland crops showed a grain yield increase on winter (by 14.0%) and spring cereals (by 9.5%) and also on legumes (by 6.6%). Inoculation with selected strains of *Azospirillum* sp. increased crop yields and enhanced the efficacy in plant nutrient availability [23]. The inoculation of maize seeds with *Azospirillum brasilense* intensified the plant growth, improved biochemical traits, and raised the nitrogen use efficiency under a nitrogen deficit [47]. Little effect of inoculation with *Azospirillum brasilense* on grain yield was observed for wheat (*Triticum aestivum*) [48]. A beneficial influence of co-inoculation of *Azospirillum lipoferum* and *Baccilus megaterium* was observed for providing balanced N and P nutrition for wheat plants [49].

The research on *Azotobacter chroococcum* spp. in crop production has manifested its significance in plant nutrition and its contribution to soil fertility. The *Azotobacteria* genus synthesizes auxins, cytokinins, and GA–like (Gibberellin-like) substances, and these growth materials are the primary substances controlling enhanced growth. These hormonal substances affect the growth of the closely associated higher plants [50].

5. Conclusions

The results obtained in several locations proved that it was possible to reduce the fertilization of sugar beet with mineral nitrogen by 30% without reducing the biological yield of sugar and pure sugar yield, and even to obtain an increase as a result of the use of the Penergetic-K and Penergetic-P growth activators and the Azoter preparation containing the bacteria *Azotobacter chroococcum*, *Azospirillum brasilense*, and *Bacillus megaterium*. This proved that high sugar yields can be obtained with more environmentally friendly technologies.

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