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ORIGINAL PAPER

Effect of herbicide and biostimulant treatment strategies on the contents of minerals in soybean seeds*

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Abstract

A way to alleviate various abiotic stresses in plants is to use biostimulants, as they improve photosynthetic efficiency, plant growth and root development, thereby facilitating the uptake of nutrients. The aim of this study was to assess the effect of biostimulant application with concomitant various chemical weed control strategies on the contents of selected macro- and micronutrients in soybean (*Glycine max* (L.) Merr.) seeds. A field experiment with soybean cultivation was conducted in 2020-2022 at the Experimental Station in Czesławice belonging to the University of Life Sciences in Lublin (Poland). The first experimental factor considered was the herbicide protection: (A) no herbicides, (B) soil herbicide Boxer 800 EC (prosulfocarb), (C) foliar herbicide Corum 502.4 SL (bentazone, imazamox) + Dash HC adjuvant (methyl oleate, fatty alcohol), and (D) soil herbicide Boxer 800 EC + foliar herbicide Corum 502.4 SL + Dash HC adjuvant. The second experimental factor was the biostimulant type: (a) no biostimulant, (b) Asahi SL (sodium para-nitrophenolate, sodium ortho-nitrophenolate, sodium 5-nitroguaiacolate), (c) Aminoplant (organic nitrogen, ammonia nitrogen, free amino acids, organic carbon), and (d) Kelpak SL (auxins, cytokinins). Herbicide protection entailing the use of Boxer 800 EC followed by Corum 502.4 SL (D) exerted the most beneficial effect on the contents of nitrogen and magnesium in soybean seeds. Phosphorus accumulation in the seeds was promoted only by the soil herbicide Boxer 800 EC applied immediately after seed sowing, while suppressed by the application of the foliar herbicide Corum 502.4 SL. Among the biostimulants tested, Kelpak SL from *Ecklonia maxima* algae elicited the most beneficial effect on the nitrogen, phosphorus and calcium contents in soybean seeds. In turn, the Aminoplant biostimulant caused a significant increase in the contents of nitrogen and iron in soybean seeds compared to the no-biostimulant variant.

Keywords: *Glycine max* L., macronutrients, micronutrients, herbicides, biostimulants

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INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is a leguminous plant cultivated mainly for seeds, which are valued for their particularly high quality and used as food for humans, feed for animals, and raw material in many industries. Soybean seeds contain about 20% of fat and as much as 45% of protein, providing all the essential exogenous amino acids. In addition, they contain many valuable vitamins and minerals, including calcium, phosphorus, potassium, copper, manganese, iron and magnesium, as well as unsaturated fatty acids (Sharma et al. 2014, Biel et al. 2018, Vargas et al. 2018, Saranraj et al. 2021, Buczek et al. 2022, Gawęda et al. 2024). Studies into the effect of various agrotechnical factors on the chemical composition of seeds, including their contents of macro- and micronutrients, are of practical importance, supporting efforts to produce seeds with a high nutritional value for humans and animals.

Low soil temperature during sowing and prolonged spring colds severely hamper the cultivation of crops typical of the warm climate zones in temperate climates, as they contribute to seedling development disorders and inhibition of root growth and activity (Bradáčová et al. 2016). Such plants include soybean, which is particularly susceptible to temperature-related stress and a lack of precipitation causing water deficit in its tissues and inhibition of various physiological processes (Buczek, Jańczak-Pieniążek 2022, Tsegaw et al. 2023). One of the means to mitigate abiotic stress in plants is to use biostimulants. They are defined as organic materials and/or microorganisms applied to boost nutrient absorption, stimulate growth, as well as improve stress tolerance and crop quality. Their active substances include humic and fulvic acids, protein hydrolysates, seaweed extracts, beneficial fungi, bacteria and algae (Oosten et al. 2017). Biostimulants have been reported to positively affect the quality characteristics of crop seeds, i.e., to increase contents of elements and stimulate photosynthetic efficiency, plant growth, and root development, and therefore facilitate the uptake of nutrients (Bradáčová et al. 2016).

Besides weather conditions, the success of soybean cultivation is largely dependent on the effective elimination of weeds, which is feasible by applying properly selected herbicides. The slow initial growth of soybean plants and their weak competition with weeds for water and nutrients (Gawęda et al. 2020, Tehulie et al. 2021) result in fewer amounts of macro- and micronutrients accumulated in their seeds (Harre, Young 2020). However, the use of herbicides prior to emergence may adversely affect the germination capacity of soybean seeds, whereas their post-emergence application may cause damage to plants (Poston et al. 2008). Hence, herbicides may negatively affect soybean development, root nodulation and nitrogen fixation, and thereby also the seed yield quality parameters, including the content of elements (Steppig et al. 2019, Ribeiro et al. 2021). A solution in this case may be the use of biostimulants supporting plant regeneration by increasing the chlorophyll content, which may decrease significantly upon herbicide treatment (Franzoni et al. 2023). Ample studies conducted so far have demonstrated beneficial effects of the combined use of appropriately selected biostimulants and herbicides on the yield quality parameters. These effects were attributed to the successful weed control resulting in the improvement of crop growth conditions and to plant development and nutrient uptake promotion by these preparations (Soltani et al. 2015, Kanatas et al. 2022, Franzoni et al. 2023, Zarzecka et al. 2024).

Owing to the growing popularity of soybean cultivation in Poland and often unfavourable weather conditions for its growth and development in this area, a field experiment was conducted to investigate the effect of various herbicides and biostimulants on the contents of selected macro- and micronutrients in soybean seeds. It was hypothesized that the use of biostimulants as preparations mitigating the adverse effects of various stress factors disturbing the vital processes of plants would have a positive impact on the contents of selected macro- and microelements in soybean seeds, also under the conditions of varied chemical weed control.

MATERIALS AND METHODS

Localization and scheme of the experiment

A field experiment with the cultivation of soybean cv. Lajma was performed in 2020-2022 at the Experimental Station in Czesławice belonging to the University of Life Sciences in Lublin, Poland (51°18′23″N, 22°16′1″E). The two-factor experiment was conducted in a randomized block design with three replications, on plots with the surface area of 24 m^2 (4 × 6 m). The first experimental factor was the herbicide treatment: (A) no herbicides – mechanical weed control only, (B) herbicide Boxer 800 EC (prosulfocarb) – applied at a dose of 3.5 dm^3 ha^{-1}, (C) herbicide Corum 502.4 SL (bentazone, imazamox) – applied in a dose of 1.25 dm^3 ha^{-1} + Dash HC adjuvant (methyl oleate, fatty alcohol) – applied in a dose of 1 dm^3 ha^{-1}, and (D) herbicide Boxer 800 EC – applied in a dose of 3.5 dm^3 ha^{-1} and herbicide Corum 502.4 SL – applied in a dose of 1.25 dm^3 ha^{-1} + Dash HC adjuvant – applied in a dose of 1 dm^3 ha^{-1}. The second experimental factor was the biostimulant treatment: (a) no biostimulant, (b) Asahi SL (sodium para-nitrophenolate, sodium ortho-nitrophenolate, sodium 5-nitroguaiacolate), (c) Aminoplant – containing free amino acids and short peptide chains (organic nitrogen, ammonium nitrogen, free amino acids, organic carbon), and (d) Kelpak SL – an extract from *Ecklonia maxima* algae (auxins, cytokinins).

During the soybean vegetation period, all biostimulants were applied twice: at the stage of the unfolded trifoliate leaf on the third node (BBCH 13) and at the beginning of flowering (BBCH 61). In both terms, the biostimulants were applied in the following doses: Asahi SL – 0.5 dm^3 ha^{-1}, Aminoplant – 1.5 dm^3 ha^{-1}, and Kelpak SL – 2.5 dm^3 ha^{-1}. Doses and application dates of herbicides and biostimulants were determined based on the recommendations included on the labels on the plant protection products.

Typical plough tillage was performed throughout the experiment, and harrowing was performed as a maintenance treatment at the stage of the third trifoliate leaf on the second node (BBCH 12).

Each year, soybean seeds were sown after spring wheat in the first ten days of May, at a density of 70 seeds per 1 m^2, depth of 3 cm, and row spacing of 22.5 cm. Before sowing, soybean seeds were inoculated with *Bradyrhizobium japonicum* bacteria, and mineral fertilization was applied in the following doses: N – 30 kg ha^{-1} (34.5% ammonium nitrate), P_2O_5 – 40 kg ha^{-1} (40% superphosphate), K_2O – 80 kg ha^{-1} (60% potassium salt).

In each year of the experiment, soybeans were harvested in the first ten days of September, when the seeds were fully ripe (BBCH 89).

Soil and weather conditions

The experiment was established on loess soil, belonging to a good wheat complex and quality class II in the Polish soil taxonomy. The content of phosphorus (P), potassium (K) and magnesium (Mg) in the arable soil layer (25 cm) was 130.5 mg kg^{-1}, 176.6 mg kg^{-1} and 67.6 mg kg^{-1}, respectively The humus content was 1.4%, and the soil pH was neutral (pH in 1 mol KCl – 7.2).

All soybean growing seasons in the 2020-2022 study period were characterized by higher total precipitation than in the multi-year period (Figure 1). The highest sum of precipitation was recorded in 2020, especially in May,

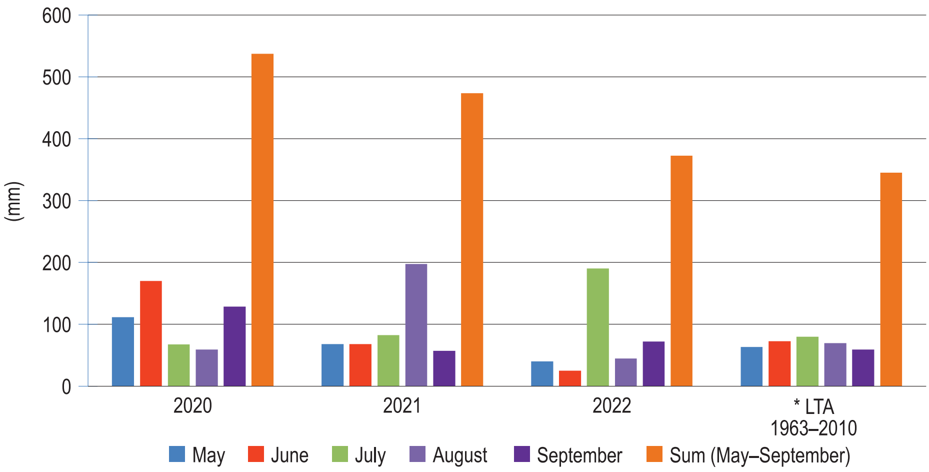

Fig. 1. Total precipitations (mm) in the growing season of soybean, recorded by the Meteorological Station in Czesławice. * LTA – long-term averages

June and August, whereas the lowest one occurred in the growing season in 2022. At the time of sowing and initial soybean growth (May, June) and in August, the amount of precipitation was significantly lower than in the respective periods of the other study years and the multi-year period. A higher average temperature was recorded for all soybean growing seasons (May-September) compared to the multi-year period (Figure 2). The warmest

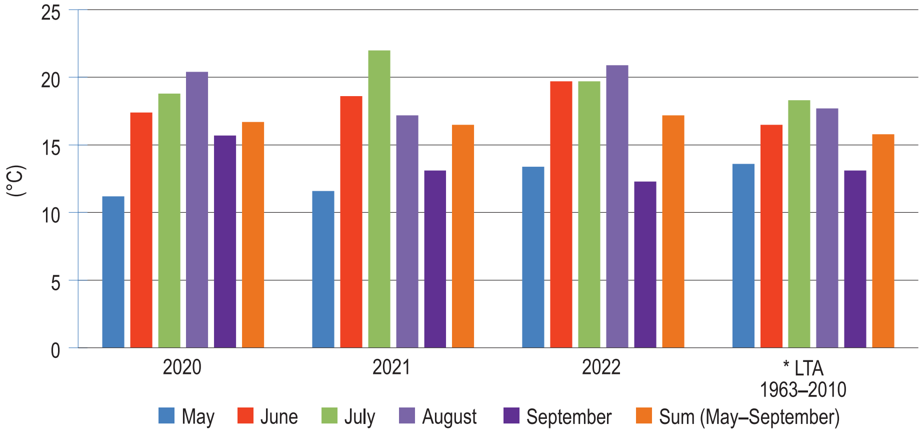

Fig. 2. Air temperature (°C) in the growing season of soybean, recorded by the Meteorological Station in Czesławice. * LTA – long-term averages

turned out to be the 2022 season, during which the air temperature was the lower than in the corresponding month of the multi-year period and the remaining study years only in August. In 2020, in the month of soybean sowing (May), the average air temperature was the lowest among the study years and compared to the multi-year period.

Scope of study and statistical analysis

In order to determine the content of elements, soybean seeds were wet-mineralized in a mixture of nitric (V) and chloric (VII) acids. The total nitrogen (N) of the seeds was determined with the Kjeldahl method (PN-EN ISO 5983-1:2006: Feeds – Determination of nitrogen content and calculation of total protein content – Part 1: Kjeldahl method). Contents of the other mineral components were determined via the inductively coupled plasma atomic emission spectrometry method – ICP-AES (PN-EN 15510:2017-09). Analyses were carried out using an ASA Atomic Absorption Spectrometer (Thermo Fisher Scientific ICE 3000 series, Waltham, Massachusetts, USA).

The research results collected in 2020-2022 were subjected to the analysis of variance (ANOVA), and the significance of differences was estimated using the Tukey test at a significance level of $p \leq 0.05$. The effect of herbicides, biostimulants and study years and their interactions on the content of selected macro- and micronutrients in soybean seeds was determined. The Statistica 13.1 software was used for calculations.

RESULTS AND DISCUSSION

The data presented in Table 1 indicates that the herbicide treatment had a significant effect on the contents of nitrogen, phosphorus and magnesium in soybean seeds. In turn, the biostimulants diversified the contents of nitrogen, phosphorus, calcium and iron. The contents of all determined macronutrients differed significantly depending on weather conditions in individual growing seasons. The herbicides × study years interaction significantly modified the contents of nitrogen, phosphorus, magnesium and calcium, whereas the biostimulants × study years interaction had a significant effect on the contents of nitrogen and phosphorus in soybean seeds. In turn, the herbicides × biostimulants interaction had a significant effect only on the contents of nitrogen and phosphorus, while the contents of the analyzed macro- and micronutrients remained unaffected by the herbicides × biostimulants × study years interaction.

Table 1

Significance of effects in ANOVA for macro- and micronutrient content of soybean seeds

Specification	H	B	Y	H x B	H x Y	B x Y	H x B x Y
Macronutrients							
N	***	***	***	***	***	***	ns
P	***	***	***	***	***	***	ns
K	ns	ns	***	ns	ns	ns	ns
Mg	*	ns	***	ns	***	ns	ns
Ca	ns	***	***	ns	**	ns	ns
Micronutrients							
Fe	ns	**	ns	ns	ns	ns	ns
Mn	ns	ns	ns	ns	ns	ns	ns
Zn	ns	ns	ns	ns	ns	ns	ns

H – herbicides, B – biostimulants, Y – years significant effects: *** significance level at $p \leq 0.001$, ** significance level at $p \leq 0.01$, * significance level at $p \leq 0.05$, ns – not significant.

The type of herbicides used significantly differentiated the contents of nitrogen, phosphorus and magnesium in soybean seeds (Table 2). On average, over three study years, the highest nitrogen content was determined in the seeds from plot D, where herbicide Boxer 800 EC (prosulfocarb) was used in combination with the subsequent application of Corum 502.4 SL (bentazone, imazamox). Compared to plot D, the content of this macroelement was by 13.7% lower in the seeds from the control plot (A), by 12.2% in those from the plots with the soil herbicide (B) and by 5.69% in those from the plots with the foliar herbicide (C). The coupled herbicide protection (plot D) was also found to promote the greatest magnesium accumulation in soybean

Table 2

Content of macronutrients in soybean seeds depending on herbicides, biostimulants (mean for 2020-2022), and experimental year

Specification	N ($g\ kg^{-1}$)	P ($g\ kg^{-1}$)	K ($g\ kg^{-1}$)	Mg ($g\ kg^{-1}$)	Ca ($g\ kg^{-1}$)
Herbicides					
A (no herbicides)	48.97 c	8.521 *a*	18.63 *a*	2.356 b	0.725 *a*
B (Boxer 800 EC)	49.83 c	8.419 *a*	18.69 *a*	2.363 *a*b	0.710 *a*
C (Corum 502.4 SL)	53.50 b	7.835 b	18.40 *a*	2.384 *a*b	0.712 *a*
D (Boxer 800 EC and Corum 502.4 SL)	56.73 *a*	7.854 b	18.28 *a*	2.433 *a*	0.708 *a*
Biostimulants					
No biostimulant	50.11 *b*	7.883 *b*	18.43 *a*	2.387 *a*	0.707 *bc*
Asahi SL	50.16 *b*	7.876 *b*	18.58 *a*	2.359 *a*	0.690 *c*
Aminoplant	54.85 *a*	8.081 *b*	18.46 *a*	2.396 *a*	0.724 *ab*
Kelpak SL	53.91 *a*	8.789 *a*	18.52 *a*	2.394 *a*	0.734 *a*
Years					
2020	54.41 *a*	8.357 *b*	20.76 *a*	2.321 *b*	0.607 *b*
2021	54.45 *a*	7.233 *c*	21.03 *a*	2.354 *b*	0.609 *b*
2022	47.91 *b*	8.883 *a*	13.71 *b*	2.477 *a*	0.926 *a*

Different letters within the column denote significant differences ($p \leq 0.05$). The same letter means lack of significantly different values ($p \leq 0.05$).

seeds. However, its content in the seeds from this plot was significantly higher only compared to the seeds from the non-treated plot (A). Similarly to the present study, Harre and Young (2020) showed the highest accumulation of elements (N, P, K) in soybean seeds after crop treatment with the most effective complex chemical protection including pre- and post-emergence herbicides. The higher content of macronutrients in the seeds harvested from the weed-free plots may be due to the absence of crop competition with weeds for nutrients, which facilities their accumulation in plants. In contrast, in the experiment conducted by Macák et al. (2015), significantly higher contents of potassium and magnesium were obtained in the seeds of peas and cereals from a no-pesticide cultivation variant compared to the conventional cultivation with herbicides and other plant protection agents.

Intensive chemical weed control consisting in either the use of both soil and foliar herbicides (D) or in the exclusive application of the foliar herbicide (C) did not enhance phosphorus accumulation in soybean seeds (Table 2). In the treatment without herbicides, the phosphorus content was much higher than that determined in the seeds from treatments C and D, i.e., by 8.76 and 8.49%, respectively. Similar results were reported by Rani and Kapoor (2024), who determined a reduced phosphorus content of onion upon the use of chemical plant protection agents compared to organic cultivation without pesticides. However, these authors found a similar relationship in the case of potassium content of pea seeds and onion, while in our study, herbicide protection had no effect on the content of this macroelement.

The herbicides used did not significantly differentiate the contents of micronutrients determined in soybean seeds (Figures 3, 4, 5). Similarly, Biel

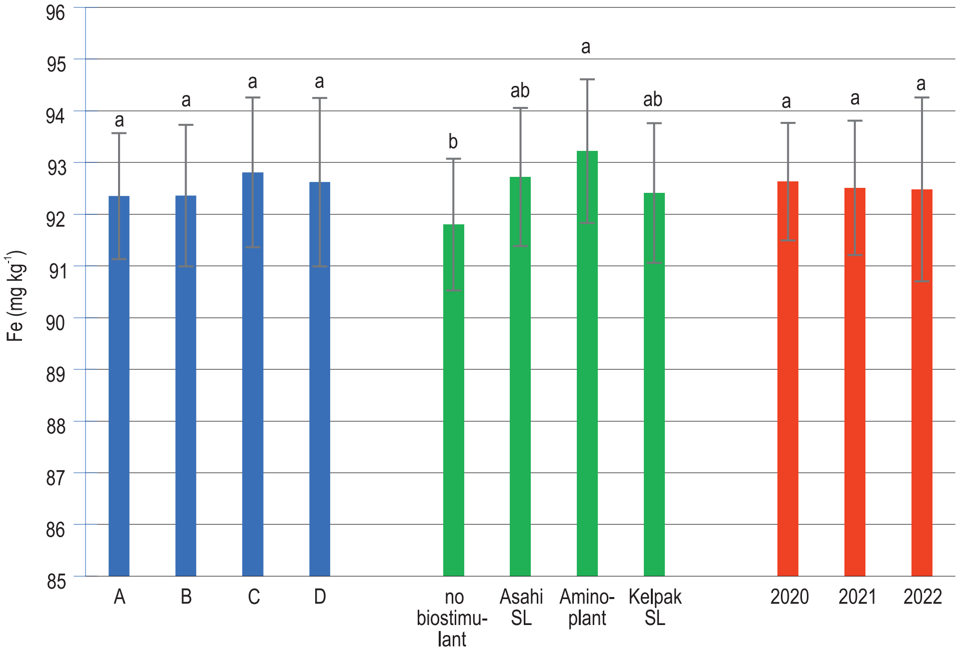

Fig. 3. Content of iron in soybean seeds depending on herbicides, biostimulants (mean for 2020-2022), and experimental year. A – no herbicides, B – Boxer 800 EC, C – Corum 502.4 SL, D – Boxer 800 EC and Corum 502.4 SL

et al. (2018) found no significant differences in manganese content under the influence of herbicides used in soybean cultivation. In contrast, Rani and Kapoor (2024) showed higher contents of Fe and Zn in sesame, millet and potatoes when no plant protection agents, including herbicides, were used compared to the conventional cultivation with pesticide application.

Reports by many authors have proven that biostimulants have a beneficial effect on plant metabolism, enhancing the activity and synthesis of phytohormones, which stimulates their growth and development, and at the same time contributes to better uptake, translocation and use of macro- and microelements (Pacholczak et al. 2015, Kocira et al. 2017, Abdel-Gawad, Youssef 2019, Yang et al. 2024). In the present study, the biostimulant treat-

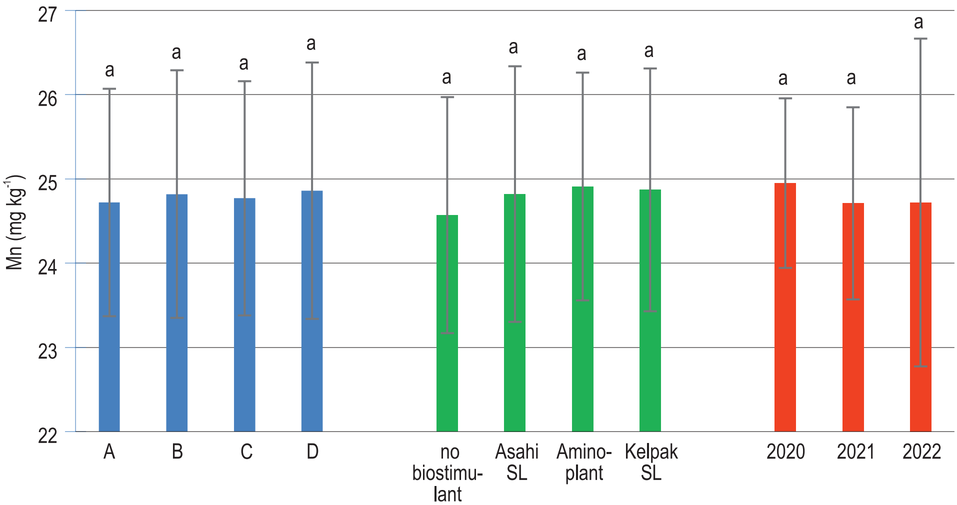

Fig. 4. Content of manganese in soybean seeds depending on herbicides, biostimulants (mean for 2020-2022), and experimental year. Explanations as in Fig. 3

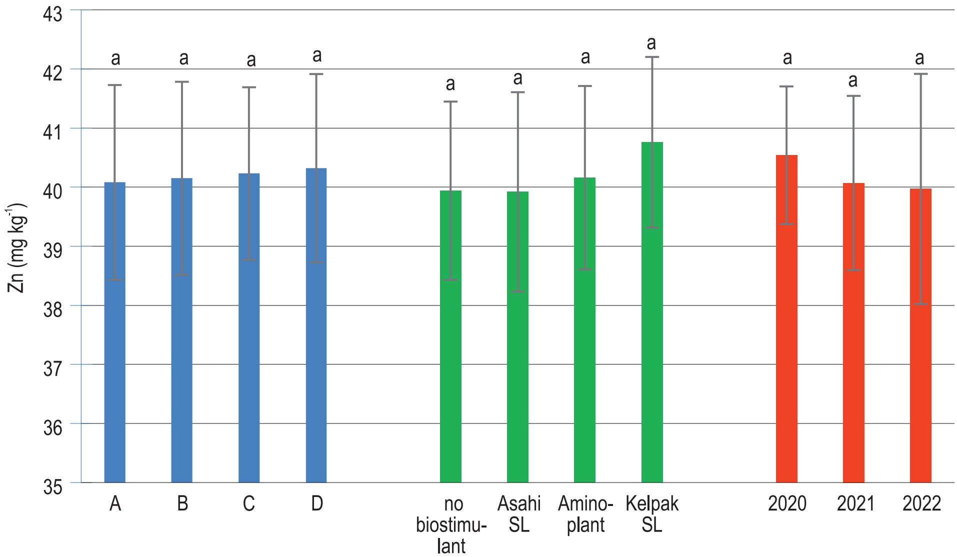

Fig. 5. Content of zinc in soybean seeds depending on herbicides, biostimulants (mean for 2020-2022), and experimental year. Explanations as in Fig. 3

ments modified the contents of nitrogen, phosphorus and calcium in soybean seeds (Table 2). After the application of Aminoplant and Kelpak biostimulants, the nitrogen content of the seeds was significantly higher than in those harvested from the unamended plot, by 9.46 and 7.58%, respectively, and by 9.35 and 7.48% compared to Asahi SL treatment. The most beneficial effect on phosphorus content in soybean seeds was observed upon the use of Kelpak SL, which caused a significant increase in its amount compared to the variants with Asahi SL and Aminoplant biostimulants and to the no-biostimulant treatment, i.e., by 11.6, 8.76 and 11.5%, respectively. In the seeds harvested from the plots amended with the other biostimulants and from the plot without biostimulant treatment, the phosphorus content was at a similar level. The Kelpak SL growth stimulant exerted the most beneficial effect also on calcium content in soybean seeds. However, the content of this macroelement was significantly higher only compared the seeds from the plots with Asahi SL treatment (by 6.38%) and those from the plots without a biostimulant (by 3.82%), while being similar to that achieved after Aminoplant treatment. Likewise in the present study, the results reported by Abbas (2013) indicate a positive effect of a seaweed extract on the nitrogen content and, to the greatest extent, on the phosphorus content of bean seeds. The beneficial effect of the seaweed extract on the contents of selected macroelements in seeds of various crop species has also been reported by other authors (Abd El- Gawad, Osman 2014, Ozaktan, Doymaz 2022). Sosnowski et al. (2014) showed an increase in phosphorus and potassium contents in alfalfa biomass after the foliar application of *E. maxima* extract compared to the control treatment, while not observing such an effect on magnesium content. In the present study, a tendency for increasing potassium and magnesium contents was found in soybean seeds upon the *Ecklonia maxima* treatment compared to the variant without biostimulant; however, the difference observed was not statistically significant. Rathore et al. (2009) proved a positive effect of the seaweed extract on the content of macronutrients (N, P and K) in soybean seeds, which was due to the improved nutrient uptake by plants in response to the presence of marine bioactive substances in the extract. Biostimulants, even those containing minerals, are not able to provide all the necessary nutrients in the amounts required by plants, but they can enhance root growth and facilitate the uptake of mineral components. The positive effect of a biostimulant containing free amino acids (Aminoplant) on the nitrogen content of soybean seeds, demonstrated in the present study, was also confirmed in the experiment conducted by Shafeek et al. (2016), who additionally reported increased contents of P and K in *Vicia faba* L. seeds after the application of a biostimulant with a high concentration of amino acids.

Weather conditions in the individual soybean growing seasons differentiated the contents of all macronutrients analyzed in the seeds (Table 2). The humid seasons of 2020 and 2021 promoted the accumulation of nitrogen and potassium in soybean seeds. On the other hand, the highest contents of phosphorus, magnesium and calcium were determined in the seeds from the 2022 growing season characterized by the highest temperature and the lowest precipitation in the months of soybean seed sowing and initial plant growth (May, June) among the study years. Also, in the study by Kozak et al. (2008), the highest phosphorus content was determined in soybean seeds in the year with exceptionally warm temperatures from flowering to seed ripening, which – according to these authors – may indicate a significant role of this component in mitigating moisture deficits in soybean development.

The statistical analysis showed that the contents of nitrogen, phosphorus, magnesium and calcium in soybean seeds differed significantly depending on the interaction of herbicide type and weather conditions in individual years of the study (Table 3).

In all study years, the most intensive herbicide protection (object D) promoted the accumulation of nitrogen in the seeds. The magnesium content was also significantly the highest under intensive herbicide protection conditions (D), but only in 2022. In the remaining years, its content was similar in

Table 3

Content of macronutrients in soybean seeds depending on the interaction of herbicides and experimental year

Years	Herbicides	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Mg (g kg^{-1})	Ca (g kg^{-1})
2020	A (no herbicides)	52.54 *cd*	8.503 *bc*	20.90 *a*	2.321 *c*	0.613 *c*
	B (Boxer 800 EC)	47.50 *e*	8.503 *bc*	20.74 *a*	2.331 *bc*	0.595 *c*
	C (Corum 502.4 SL)	58.03 *a*	7.787 *de*	20.59 *a*	2.321*c*	0.627 *c*
	D (Boxer 800 EC and Corum 502.4 SL)	59.58 *a*	8.634 *abc*	20.81 *a*	2.312*c*	0.591 *c*
2021	A (no herbicides)	52.19 *cd*	8.679 *abc*	20.99 *a*	2.322 *c*	0.607 *c*
	B (Boxer 800 EC)	59.85 *a*	7.700 *e*	21.18 *a*	2.425 *bc*	0.630 *c*
	C (Corum 502.4 SL)	51.13 *d*	6.567 f	20.95 *a*	2.332 *bc*	0.592 *c*
	D (Boxer 800 EC and Corum 502.4 SL)	54.64 *bc*	5.984 f	21.01 *a*	2.337 *bc*	0.605 *c*
2022	A (no herbicides)	42.18 f	8.382 *cd*	14.00 *a*	2.424 *bc*	0.955 *a*
	B (Boxer 800 EC)	42.14 f	9.053 *ab*	14.15 *a*	2.332 *bc*	0.904 *b*
	C (Corum 502.4 SL)	51.35 *d*	9.152 *a*	13.66 *a*	2.500 *ab*	0.916 *ab*
	D (Boxer 800 EC and Corum 502.4 SL)	55.97 *b*	8.943 *abc*	13.01 *a*	2.650 *a*	0.928 *ab*

Explanations as in Table 2.

the seeds from all experimental variants. In turn, the lowest phosphorus content was determined in soybean seeds harvested in 2021 from plots C and D. In 2022, its content was higher in the seeds harvested from plots with the herbicides Boxer 800 EC (B) and Corum 502.4 SL (C) than in those from the plot without chemical weed control (A). The highest calcium content was determined in the seeds from all plots in 2022, and differed significantly from that found in the other study years, regardless of a weed control strategy.

The study years × biostimulant interaction significantly differentiated the nitrogen and phosphorus contents of soybean seeds (Table 4). In 2020,

Table 4

Content of macronutrients in soybean seeds depending on the interaction of biostimulants and experimental year

Years	Biostimulants	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Mg (g kg^{-1})	Ca (g kg^{-1})
2020	no biostimulant	51.87 *cd*	8.140 *de*	20.52 *a*	2.340 *a*	0.607 *a*
	Asahi SL	53.46 *cd*	7.754 *f*	20.88 *a*	2.280 *a*	0.582 *a*
	Aminoplant	57.68 *ab*	8.458 *cd*	20.78 *a*	2.350 *a*	0.625 *a*
	Kelpak SL	54.64 *bc*	9.075 *ab*	20.85 *a*	2.315 *a*	0.613 *a*
2021	no biostimulant	51.42 *de*	6.831 *g*	20.81 *a*	2.341 *a*	0.600 *a*
	Asahi SL	51.45 *de*	6.897 *g*	21.15 *a*	2.315 *a*	0.593 *a*
	Aminoplant	58.24 *a*	7.403 *fg*	20.97 *a*	2.368 *a*	0.622 *a*
	Kelpak SL	56.70 *ab*	7.799 *ef*	21.18 *a*	2.392 *a*	0.619 *a*
2022	no biostimulant	47.04 *f*	8.679 *bcd*	13.95 *a*	2.480 *a*	0.913 *a*
	Asahi SL	45.57 *f*	8.976 *abc*	13.70 *a*	2.482 *a*	0.895 *a*
	Aminoplant	48.62 *ef*	8.382 *cde*	13.64 *a*	2.471 *a*	0.926 *a*
	Kelpak SL	50.40 *de*	9.493 *a*	13.54 *a*	2.474 *a*	0.970 *a*

Explanations as in Table 2.

the highest nitrogen content was found in seeds harvested from plots amended with Aminoplant, but in 2021 – from plots with Aminoplant and Kelpak SL. In 2022, the most nitrogen was contained in grain from plots where Kelpak SL was used, but significant differences were found only in relation to plots with Aminopielik and plots without the biostimulant. In all seasons, the phosphorus content was the highest after the treatment with Kelpak SL, containing the *Ecklonia maxima* algae extract. The positive effect of seaweed extracts on plant growth, and in particular on root development, under stress conditions associated with low temperatures, and therefore on the uptake of nutrients, was demonstrated by Bradáčová et al. (2016). Thus, the use of these preparations has been found particularly viable in the cultivation of plants in warm zones of the temperate climate (Bradáčová et al. 2016).

The interaction of the experimental factors modified the nitrogen and phosphorus contents of soybean seeds (Table 5). The highest nitrogen content

Table 5

Content of macronutrients in soybean seeds depending on the interaction of herbicides and biostimulants (mean for 2020-2022)

Herbicides	Biostimulants	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Mg (g kg^{-1})	Ca (g kg^{-1})
A (no herbicides)	no biostimulant	48.53 *de*	8.125 *cdef*	18.32 *a*	2.390 *a*	0.732 *a*
	Asahi SL	45.50 *e*	8.507 *cd*	18.86 *a*	2.280 *a*	0.704 *a*
	Aminoplant	50.31 *bcd*	8.213 *cde*	18.67 *a*	2.387 *a*	0.724 *a*
	Kelpak SL	51.52 *bcd*	9.240 *ab*	18.66 *a*	2.366 *a*	0.741 *a*
B (Boxer 800 EC)	no biostimulant	50.07 *bcd*	7.685 *ef*	18.79 *a*	2.393 *a*	0.708 *a*
	Asahi SL	46.29 *e*	7.788 *def*	18.73 *a*	2.298 *a*	0.679 *a*
	Aminoplant	53.43 *b*	8.712 *bc*	18.62 *a*	2.364 *a*	0.722 *a*
	Kelpak SL	49.51 *cd*	9.489 *a*	18.63 *a*	2.397 *a*	0.731 *a*
C (Corum 502.4 SL)	no biostimulant	49.51 *cd*	7.451 *f*	18.53 *a*	2.335 *a*	0.696 *a*
	Asahi SL	49.98 *bcd*	7.788 *def*	18.48 *a*	2.367 *a*	0.693 *a*
	Aminoplant	57.77 *a*	7.698 *ef*	18.34 *a*	2.429 *a*	0.736 *a*
	Kelpak SL	56.75 *ab*	8.404 *cde*	18.26 *a*	2.407 *a*	0.722 *a*
D (Boxer 800 EC and Corum 502.4 SL)	no biostimulant	52.31 *bc*	8.272 *cde*	18.09 *a*	2.431 *a*	0.690 *a*
	Asahi SL	58.86 *a*	7.420 *f*	18.25 *a*	2.490 *a*	0.684 *a*
	Aminoplant	57.87 *a*	7.700 *ef*	18.21 *a*	2.405 *a*	0.715 *a*
	Kelpak SL	57.87 *a*	8.023 *cdef*	18.55 *a*	2.407 *a*	0.743 *a*

Explanations as in Table 2.

was determined in the seeds harvested from plots amended with prosulfocarb before plant emergence in combination with the subsequent application of bentazone and imazamox (D) and biostimulants: Asahi SL, Aminoplant or Kelpak SL. A similar content of this macroelement was assayed in soybean seeds harvested from plot C after Aminoplant and Kelpak SL treatment. The lowest nitrogen content was determined in the seeds from plots after the application of Asahi SL but without chemical weed control or from those treated with Boxer 800 EC herbicide (B). The phosphorus content was the highest in soybean seeds form the plots with the soil herbicide Boxer 800 EC (B) and Kelpak SL biostimulant treatment. The use of Kelpak SL had the most beneficial effect on the content of this macroelement in seeds from plots A, B and C. In the variant with the most intensive chemical protection (D), the highest phosphorus content was determined only in the seeds from the no-biostimulant treatment and slightly lower upon Kelpak SL treatment. The results of the present study, indicating in most cases a greater accumulation of nitrogen and phosphorus in the seeds from plots amended with biostimulants, confirm the theory that these preparations increase the absorption of minerals and the efficiency of nutrient use in plants (Calvo et al. 2014, Du Jardin 2015, Popko et al. 2018).

Among the micronutrients determined, the use of biostimulants significantly differentiated only the iron content of soybean seeds (Figures 3, 4, 5). The use of Aminoplant containing free amino acids had the most beneficial effect on its accumulation, as its content was significantly higher by 1.55% compared to the seeds from the plot without the biostimulant treatment. Also, the Asahi SL and Kelpak SL preparations increased the content of this micronutrient, but the differences observed were statistically insignificant.

Treatments with all the biostimulants tended to increase the manganese content, whereas Aminoplant and Kelpak SL also affected the zinc content of soybean seeds. In turn, Ozaktan and Doymaz (2022) observed a significant increase in the Fe and Zn contents in *Phaseolus vulgaris* L. seeds upon the use of a biostimulant from marine algae. The beneficial effect of the foliar application of the *K. alvarezii* extract on the content of microelements in mung bean seeds was demonstrated by Zodape et al. (2010), i.e., a 10% extract of this marine algal species caused a significant increase in the content of manganese (by 36.8%) and iron (by 16.1%) and, to a negligible extent, also of zinc (by 1.83%) compared to the control variant. On the other hand, in the study by Sosnowski et al. (2014), the spraying with a seaweed extract increased contents of zinc and manganese in the aerial biomass of alfalfa, whereas a two-fold increase in the iron content of Abelina soybean seeds was reported by Szparaga et al. (2021) after the foliar application of *L. officinale* extract. However, the latter authors showed no significant respective correlation for the zinc content of soybean seeds.

Weather conditions in the individual growing seasons of soybean did not significantly differentiate the contents of iron, manganese and zinc in its seeds (Figures 3, 4, 5). Their contents were slightly higher only in the 2020 growing season, characterized by the highest total precipitation among the study years.

The statistical analysis also showed no significant differences in the contents of micronutrients in soybean seeds depending on the interaction

Table 6

Content of micronutrients in soybean seeds depending on the interaction of herbicides and experimental year

Years	Herbicides	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})
2020	A (no herbicides)	92.56 *a*	24.88 *a*	40.64 *a*
	B (Boxer 800 EC)	92.27 *a*	24.84 *a*	40.52 *a*
	C (Corum 502.4 SL)	92.91 *a*	25.01 *a*	40.38 *a*
	D (Boxer 800 EC and Corum 502.4 SL)	92.77*a*	25.08 *a*	40.63 *a*
2021	A (no herbicides)	92.33 *a*	24.62 *a*	40.07 *a*
	B (Boxer 800 EC)	92.30 *a*	24.74 *a*	39.90 *a*
	C (Corum 502.4 SL)	92.80 *a*	24.67 *a*	40.15 *a*
	D (Boxer 800 EC and Corum 502.4 SL)	92.60 *a*	24.81 *a*	40.18 *a*
2022	A (no herbicides)	92.16 *a*	24.67 *a*	39.54 *a*
	B (Boxer 800 EC)	92.52 *a*	24.89 *a*	40.03 *a*
	C (Corum 502.4 SL)	92.74 *a*	24.63 *a*	40.17 *a*
	D (Boxer 800 EC and Corum 502.4 SL)	92.51 *a*	24.70 *a*	40.15 *a*

Explanations as in Table 2.

Table 7

Content of micronutrients in soybean seeds depending on the interaction of biostimulants and experimental year

Years	Biostimulants	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})
2020	no biostimulant	91.89 *a*	24.54 *a*	40.33 *a*
	Asahi SL	92.77 *a*	25.05 *a*	40.34 *a*
	Aminoplant	93.31 *a*	25.16 *a*	40.48 *a*
	Kelpak SL	92.53 *a*	25.06 *a*	41.02 *a*
2021	no biostimulant	91.82 *a*	24.50 *a*	39.76 *a*
	Asahi SL	92.77 *a*	24.79 *a*	39.82 *a*
	Aminoplant	93.09 *a*	24.77 *a*	40.08 *a*
	Kelpak SL	92.35 *a*	24.77 *a*	40.64 *a*
2022	no biostimulant	91.68 *a*	24.68 *a*	39.73 *a*
	Asahi SL	92.63 *a*	24.61 *a*	39.59 *a*
	Aminoplant	93.27 *a*	24.81 *a*	39.93 *a*
	Kelpak SL	92.35 *a*	24.79 *a*	40.63 *a*

Explanations as in Table 2.

between the study years and herbicide treatments, as well as between the study years and a biostimulant type (Tables 6, 7). According to Rigo et al. (2018), the content of micronutrients in soybean seeds depends primarily

Table 8
Content of micronutrients in soybean seeds depending on the interaction of herbicides and biostimulants (mean for 2020-2022)

Herbicides	Biostimulants	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})
A (no herbicides)	no biostimulant	91.47 *a*	24.47 *a*	40.00 *a*
	Asahi SL	92.69 *a*	24.76 *a*	39.76 *a*
	Aminoplant	92.89 *a*	24.99 *a*	40.11 *a*
	Kelpak SL	92.34 *a*	24.66 *a*	40.46 *a*
B (Boxer 800 EC)	no biostimulant	91.52 *a*	24.58 *a*	39.87 *a*
	Asahi SL	92.74 *a*	24.82 *a*	39.62 *a*
	Aminoplant	92.78 *a*	24.77 *a*	40.00 *a*
	Kelpak SL	92.41 *a*	25.11 *a*	41.11 *a*
C (Corum 502.4 SL)	no biostimulant	92.07 *a*	24.55 *a*	39.77 *a*
	Asahi SL	92.68 *a*	24.82 *a*	40.11 *a*
	Aminoplant	93.99 *a*	24.93 *a*	40.22 *a*
	Kelpak SL	92.51 *a*	24.79 *a*	40.82 *a*
D (Boxer 800 EC and Corum 502.4 SL)	no biostimulant	92.12 *a*	24.69 *a*	40.12 *a*
	Asahi SL	92.78 *a*	24.86 *a*	40.17 *a*
	Aminoplant	93.22 *a*	24.96 *a*	40.32 *a*
	Kelpak SL	92.37 *a*	24.93 *a*	40.65 *a*

Explanations as in Table 2.

on the genetic constitution of soybean varieties. It is therefore only slightly influenced by other factors, including weather conditions.

The interaction of experimental factors did not significantly differentiate the micronutrients determined in soybean seeds (Table 8).

CONCLUSIONS

The present study results indicate that comprehensive weed control consisting of the use of a soil herbicide combined with a later application of a foliar herbicide was most conducive to the accumulation of nitrogen and magnesium, but hampered the accumulation of phosphorus in soybean seeds. The nitrogen content of the seeds from this variant was the highest when the herbicide treatment was aided by a biostimulant application.

Among the herbicide protection variants tested, the most beneficial for phosphorus accumulation in soybean seeds was the exclusive use of the soil herbicide Boxer 800 EC (prosulfocarb).

The study results also proved the advisability of using biostimulants from marine algae and containing amino acids in soybean cultivation because of their positive impact on the accumulation of elements, macronutrients in particular.

The Kelpak SL preparation from *Ecklonia maxima* algae ensured the highest nitrogen, phosphorus and calcium contents of soybean seeds. Also, the application of the Aminoplant biostimulant containing free amino acids caused a significant increase in the nitrogen content of the seeds compared to the seeds harvested from the no-biostimulant plot. Aminoplant was also the only tested growth stimulant tested which significantly increased the iron content of the seeds.

Soybean growing seasons characterized by greater precipitation facilitated the accumulation of nitrogen and potassium in the seeds. In contrast, the seeds harvested in the season with the highest temperature and the lowest precipitation had significantly the highest contents of phosphorus, magnesium, and calcium.

In conclusion, it can be stated that a comprehensive plant protection against weeds including the application of the soil herbicide Boxer 800 EC (prosulfocarb) at a dose of 3.5 dm^3 ha^{-1} followed by the foliar herbicide Corum 502.4 SL (bentazone, imazamox) applied in a dose of 1.25 dm^3 ha^{-1} combined with the adjuvant Dash HC (methyl oleate, fatty alcohol) applied in a dose of 1 dm^3 ha^{-1} can be recommended in soybean cultivation, especially for the purpose of obtaining seeds with high N and Mg content. The use of biostimulants such as Aminoplant (containing organic nitrogen, ammonium nitrogen, free amino acids, and organic carbon) and Kelpak SL (containing auxins and cytokinins) also has a beneficial effect on the elemental content of the seed, especially macronutrients.

Author contributions

D.G. – conceptualization, data curation, methodology, formal analysis, writing, editing, graphical preparation of results, J.B. – formal analysis, writing, graphical preparation of results, S.A. – writing, graphical preparation of results, editing, M.H. – writing, graphical preparation of results, editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest. The authors ensure that they have neither professional nor financial connections related to the manuscript sent to the Editorial Board.

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