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Application of biostimulants in the cultivation of *Helianthus tuberosus* L. on the content and uptake of iron and zinc*

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Abstract

Jerusalem artichoke (*Helianthus tuberosus* L.) is known not only for its inulin-rich tubers, but also as a potential source of micronutrients such as Fe and Zn, which play an important role in both agriculture and healthcare. Nutritional value is influenced by a variety of factors, including varieties, growing conditions, climatic conditions and the biostimulants used. An analysis of these factors is necessary to manage the cultivation of Jerusalem artichokes effectively. The aim of the study was to determine the effects of the application of biostimulants (Kaishi, Maral, Nutrigreen AD and Vanadoo) on the content and uptake of micronutrients (Fe and Zn) in the tubers of two Jerusalem artichoke varieties grown from 2021 to 2023 in Międzyrzec Podlaski. The content of selected elements was analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES). The application of the biostimulants Maral, Nutrigreen AD and Vanadoo increased the Fe content by about 10% compared to the control variant. Maral showed the greatest effect on increasing the Zn content (by 10%). The application of the individual biostimulants increased the Fe and Zn uptake. Vanadoo proved to be the best biostimulant for Zn uptake in the tested tubers. Kaishi proved to be the least effective in increasing micronutrient uptake in the JA tubers. Analysis of the two varieties Albik and Rubik showed that the Rubik variety had a higher Fe content, while the Zn content was at a similar level in both varieties. Over the three years of the trial, a decrease in the Fe content and uptake was observed, which was not the case for Zn. The decline in the Fe content in 2023 could be related to deteriorating climatic conditions, which may have affected the availability of this micronutrient to plants. The results of the study suggest that the use of biostimulants in the cultivation of Jerusalem artichokes can help to increase the micronutrient content of the tubers.

Keywords: Jerusalem artichoke, iron, zinc, biostimulants, weather conditions

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INTRODUCTION

Jerusalem artichoke *Helianthus tuberosus* L. (JA) is a plant from the Asteraceae family, which is native to North America and was originally cultivated by Native Americans (Sawicka et al. 2021). JA tubers are low in calories and have a low glycemic index. They contain about 78% water, about 2% protein and the proportion of fat is negligible. The carbohydrate content is around 17%, a large part of which is inulin (USDA 2024, Sawicka et al. 2021, Tasar and Tasar 2023). JA tubers are also rich in minerals (Sawicka et al. 2020). The nutrient content of JA, as with other crops, is the result of genetic factors, growing conditions, climatic conditions, fertilizers used, cultivation techniques and the influence of the biostimulants used (Figure 1) – Kays, Nottingham 2007, Nawaz et al. 2024, Gurmesssa et al. 2024.

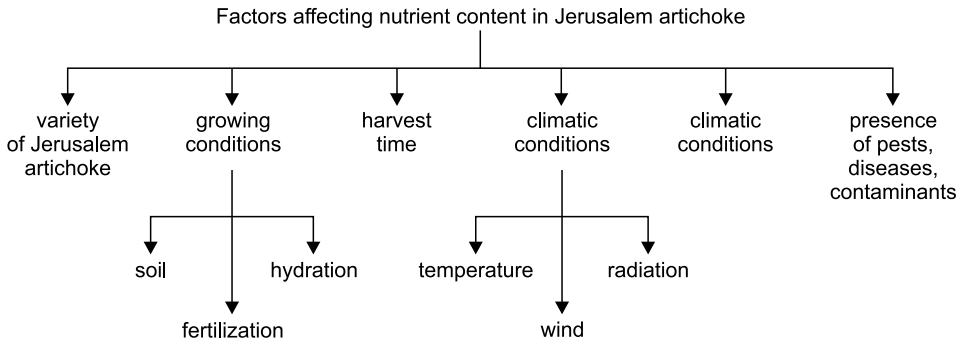


Fig.1. Factors affecting nutrient content in Jerusalem artichoke based on (Kays, Nottingham 2007)

Genetics plays an important role in determining essential traits of a plant, such as resistance to environmental stress, the ability to efficiently use minerals from the soil, and nutrient accumulation (Du et al. 2022). Numerous studies have reported that JA varieties can distinguish between macro- and micronutrient content (Barta, Patkai 2007, Sawicka, Kalembasa, 2013, Sawicka et al. 2021). JA makes efficient use of the nutrients contained in the soil (Shao et al. 2023), although the application of fertilizers is still recommended. To improve yield and tuber quality, superphosphate-enriched nitrogen and potassium fertilization is used (Amarowicz et al. 2020, Bogucka, Jankowski 2020). The ability of biostimulants to increase resource efficiency is a fundamental aspect that guarantees comparable or even higher yields than obtained from crops treated with high doses of chemical fertilizers (Ma et al. 2022). The effect of biostimulants can be associated with the increased assimilation of nitrogen, carbon and sulfur, improved photosynthetic efficiency and increased levels of carbohydrates, amino acids, proteins and phenolic compounds (Ertani et al. 2013, Jannin et al. 2013). In addition, bio-

stimulants can strengthen the immune system of plants and provide better protection against biotic and abiotic stress factors such as heat, cold, frost, mechanical and chemical stress, salinity, drought and oxidative stress (Park et al. 2017, Van Oosten et al. 2017, Yan et al. 2022). However, JA has a high tolerance and adaptability to high and low air temperatures due to fructan metabolism (Jiao et al. 2018, Zhao et al. 2021, Boonmahome et al. 2023). The plant easily adapts to different growing conditions and is successfully cultivated in many climates such as the Mediterranean, tropics and temperate monsoon areas, and at different levels of shading (Shen et al. 2021). Nevertheless, extreme temperatures can affect plant growth and development, which may influence the final chemical composition of the tubers. In dry and cool conditions, Jerusalem artichoke tubers can be susceptible to frost damage as they tend to dry out on the soil surface. The amount of available water is crucial for the development of the plant and influences the chemical composition of the plant. Both water deficiency and excess water can negatively affect the nutrient content (Rodrigues et al. 2007, Puangbut et al. 2015, Gao et al. 2018).

The content and uptake of micronutrients in Jerusalem artichoke tubers may depend on the plant variety and the biostimulants used. The aim of this study was therefore to determine the effect of foliar fertilization on the content and uptake of selected micronutrients (Fe and Zn) in two varieties of *Helianthus tuberosus* L.

MATERIALS AND METHODS

The study was conducted in the east-central region of Poland, in Międzyrzec Podlaski (51°59' N, 22°47' E), from 2021 to 2023. The experiment was set up in a two-factor arrangement (split-plot) with three replicates. The first-order factor was two Jerusalem artichoke varieties 'Albik' and 'Rubik' bred by Prof. S. Góral at the Plant Breeding and Acclimatization Institute in Radzików and entered on the list of varieties of the Central Crops Research Center (COBORU) in 1997 (Gacek 1998): cv. Albik (oval, oblong tubers, creamy skin and white flesh, tuber yield from 24 t ha⁻¹ to 34 t ha⁻¹) and cv. Rubik (egg-shaped tubers, white flesh, pink skin, yield 23 t ha⁻¹ to 40 t ha⁻¹), and the second order factor was four biostimulants: Kaishi, Maral, Nutrigreen AD, Vanadoo, and a control variant without a biostimulant. The composition of the biostimulants is shown in Table 1.

The tubers were planted at the beginning of April on sandy loam soil (Haplic Luvisol), at a distance of 30 x 65 cm on plots of 25 m² in area. Soil samples were taken and physicochemical analyses were carried out in accordance with current standards at the certified laboratory of the National Chemical and Agricultural Station in Warsaw. The soil contained the following available nutrients: average content of phosphorus, potassium and mag-

Composition of biostimulants

Biostimulator	Formulation
Kaishi	free amino acids – 12%, total N – 2%, organic N – 2%, amino acids of plant origin
Maral	total nitrogen N – 6% including organic N – 0.7%, ammonium N – 1% urea N – 4.3%, P ₂ O ₅ – 5%, K ₂ O – 5%, Zn – 1%, organic carbon – 7.5%, extract of marine algae, vitamins B1, B3, B6, alginic acid, iodine, manitol
Nutrigreen AD	total N – 8%, organic N – 8%, organic C – 23.5%, amino acids of animal origin
Vanadoo	vanadium 1.8%

nesium, acidity of 5.3 pH in KCl solution, humus content of 21.6 g kg⁻¹ and the C/N ratio of 9. Mineral fertilizers were applied each year: phosphorus fertilizer in the form of triple superphosphate 43 kg P ha⁻¹, potash fertilizer in the form of potassium salt 124 kg K ha⁻¹ and nitrogen fertilization in the form of ammonium nitrate 100 kg N ha⁻¹. The agrotechnical treatments were carried out in accordance with the requirements of good agricultural practice. No diseases or pests were detected during the growing season in any of the years of the study. The JA was harvested between 10th and 20th November; tubers missing at harvest were expected to grow aggressively next season.

The study collected data on average monthly temperatures and precipitation totals over three years. The following graphs show these values, illustrating the variability of weather conditions that affected plant growth and development during the study period (Figures 2 and 3).

The analyses were carried out at the Regional Research Center EKO-AGRO-TECH in Biala Podlaska. The Jerusalem artichoke tubers were dried to dry weight at 105°C, using the dryer weight method. The samples were then thoroughly mixed and transferred to Teflon dishes, where 6 ml of 65% nitric acid (HNO₃) and 1 ml of 36% hydrochloric acid (HCl) were added. The samples were heated in a microwave oven (Anton Paar) at the following conditions: 110°C for 5 min, 220°C for 20 min and 70°C for 25 minutes. The contents of the dishes were filtered through 150 mm sieves (Chemland) into vials, and the solution was diluted with distilled water. The solution was analyzed using an ICP-OES spectrometer (Spectroblue EOP, Ametek), according to LST EN 15510:2017. The elemental contents of Fe and Zn were quantified with reference to the calibration curve of a multi-element standard solution (VHG, Standard, LGC) in their linear range. Micronutrient intake was calculated as the product of JA tuber dry weight yield and the content of individual elements (Fe, Zn).

The results were statistically evaluated using the analysis of variance (ANOVA). The significance of the sources of variation was assessed using

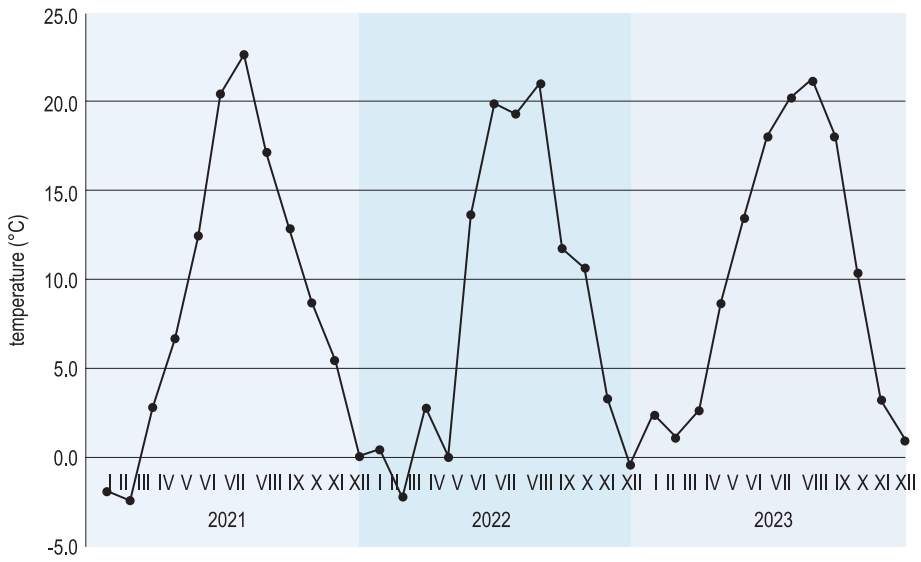


Fig.2. Average air temperature in 2021-2023 according to the Meteorological Station in Zawady

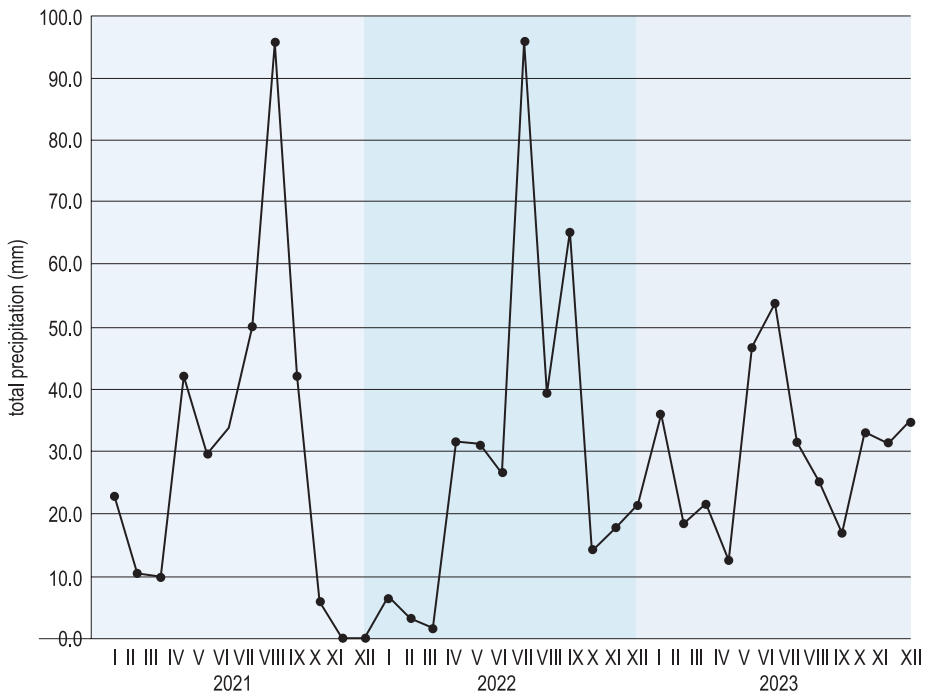


Fig. 3. Total precipitation according to the Meteorological Station in Zawady

the Fisher-Snedecor's F -test, and the significance of differences between the means of comparison was determined using the Tukey's multiple intervals at a significance level of $p \leq 0.05$. Statistical calculations were performed using a proprietary algorithm developed in Excel, following the mathematical model of Trętowski and Wojcik (1991).

RESULTS AND DISCUSSION

The average Fe content of Jerusalem artichoke tubers was 44.94 mg kg^{-1} d.m., which is consistent with the results of other researchers, 39.22 mg kg^{-1} d.m. (Harmankaya et al. 2012), 32.00 mg kg^{-1} d.m. (Judprasong et al. 2018). In contrast, Ekholm et al. (2007) reported lower values (18 mg kg^{-1} s.m.).

The average Fe content in the tubers of the control variant was 41.85 mg kg^{-1} s.m. (Figure 4a). As a result of the application of biostimulants, no significant differences in the Fe content were found between the variants compared. However, the biostimulants Maral, Nutrigreen AD and Vanadoo increased the Fe content by about 10% compared to the control, while the biostimulant Kaishi increased it by 4%. The study also determined the Fe uptake by the tubers, which amounted to 351.96 g ha^{-1} d.m. and was higher than in the study by Németh and Izsáki (2006), in which the uptake at the maximum of green yield was 280.00 g ha^{-1} d.m. The application of each biostimulant resulted in a significant increase (by 17-29%) in Fe uptake by JA tubers relative to the control variant (Figure 4b).

The average Zn content of Jerusalem artichoke tubers was 20.53 mg kg^{-1} d.m. Lower results were obtained in studies by other authors 13.56 mg kg^{-1} d.m. (Harmankaya et al. 2012), 14.00 mg kg^{-1} d.m. (Ekholm et al. 2007). The average Zn content of Jerusalem artichoke tubers was 19.37 mg kg^{-1} d.m. in the control variant. Although the differences in the Zn content were insignificant, it is worth noting that plants treated with the biostimulant Maral had a 10% higher average content of this element, while the other biostimulants raised its content by 4-7% (Figure 4a).

The average Zn uptake was 148.48 g ha^{-1} s.m., while another study yielded 180 g ha^{-1} d.m. (Németh, Izsáki 2006). The application of any of the biostimulants increased Zn uptake (by 14-35%) compared to the control variant. In addition, Vanadoo increased the uptake of the element significantly compared to the other biostimulants.

Considering the varieties of Jerusalem artichoke, the Rubik variety was determined to have a higher Fe content (46.42 mg kg^{-1} d.m.) than the Albik variety (43.33 mg kg^{-1} d.m.). The results are lower compared to Fe values reported by other researchers. In the case of the Albik variety, Sawicka and Kalembasa (2013) reported an Fe content of $229.27 \text{ mg kg}^{-1}$ d.m., while for the Rubik variety the values ranged from $166.80 \text{ mg kg}^{-1}$ d.m. (Barta, Patkai,

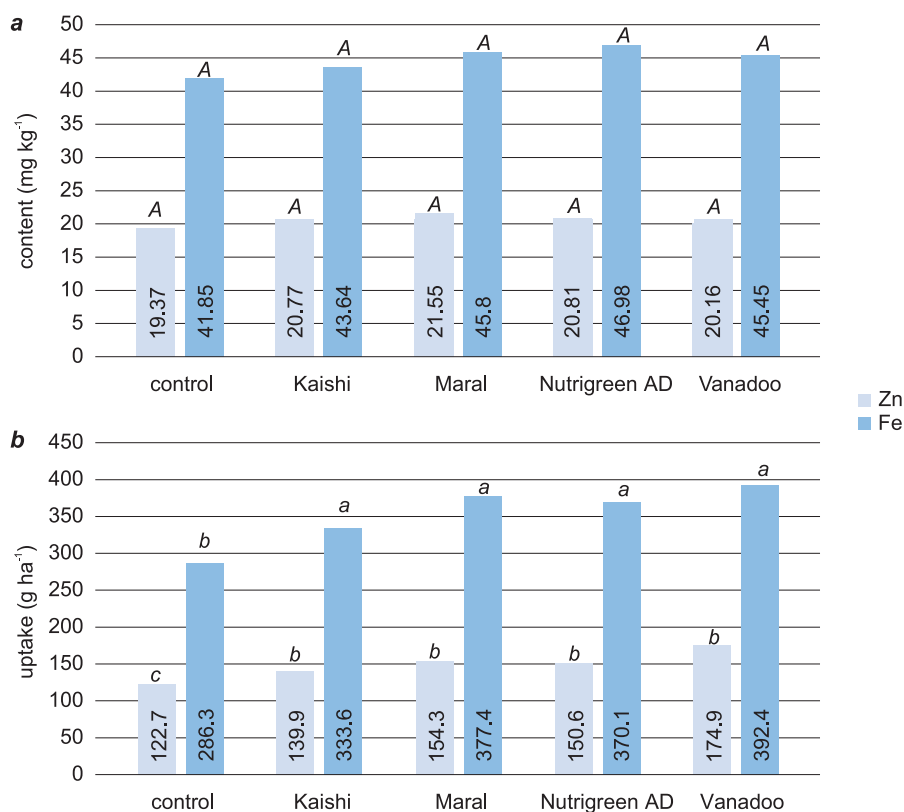


Fig. 4. Content (a) and uptake (b) of Fe and Zn in variants (control variant, Kaishi, Maral, Nutrigreen AD, Vanadoo). Significantly different value are marked with different letters above bars (separately *a-b*) at $p < 0.05$

2007) to 227.57 mg kg⁻¹ d.m. (Sawicka, Kalembasa 2013). Differences in the Fe content may be due to different climatic conditions, soil mineral compositions and cultivation techniques used.

The effect of biostimulants on the Fe content was most noticeable with the Nutrigreen AD biostimulant (an increase of 14% in cv. Albik and 15% in cv. Rubik. In contrast, the Kaishi biostimulant had the weakest effect on Fe content, increasing it by 6% in cv. Albik variety and 3% in cv. Rubik (Figures 5*a-b*).

Both varieties showed similar Zn content (Albik – 20.66 mg kg⁻¹ and Rubik – 20.40 mg kg⁻¹ d.m.), Stanislawska-Glubiak and Korzeniowska (2016) indicate that the average Zn content in the Albik variety is 23.80 mg kg⁻¹ d.m. On the other hand, Barta and Patkai (2007) reported a higher level of this element in the Rubik variety, equal about 91.30 mg kg⁻¹. The variation in Zn content could be due to the varying zinc content of the soils in which Jerusalem artichoke was grown and the ambient environmental conditions. The biostimulant Maral had the greatest effect on increasing the Zn content

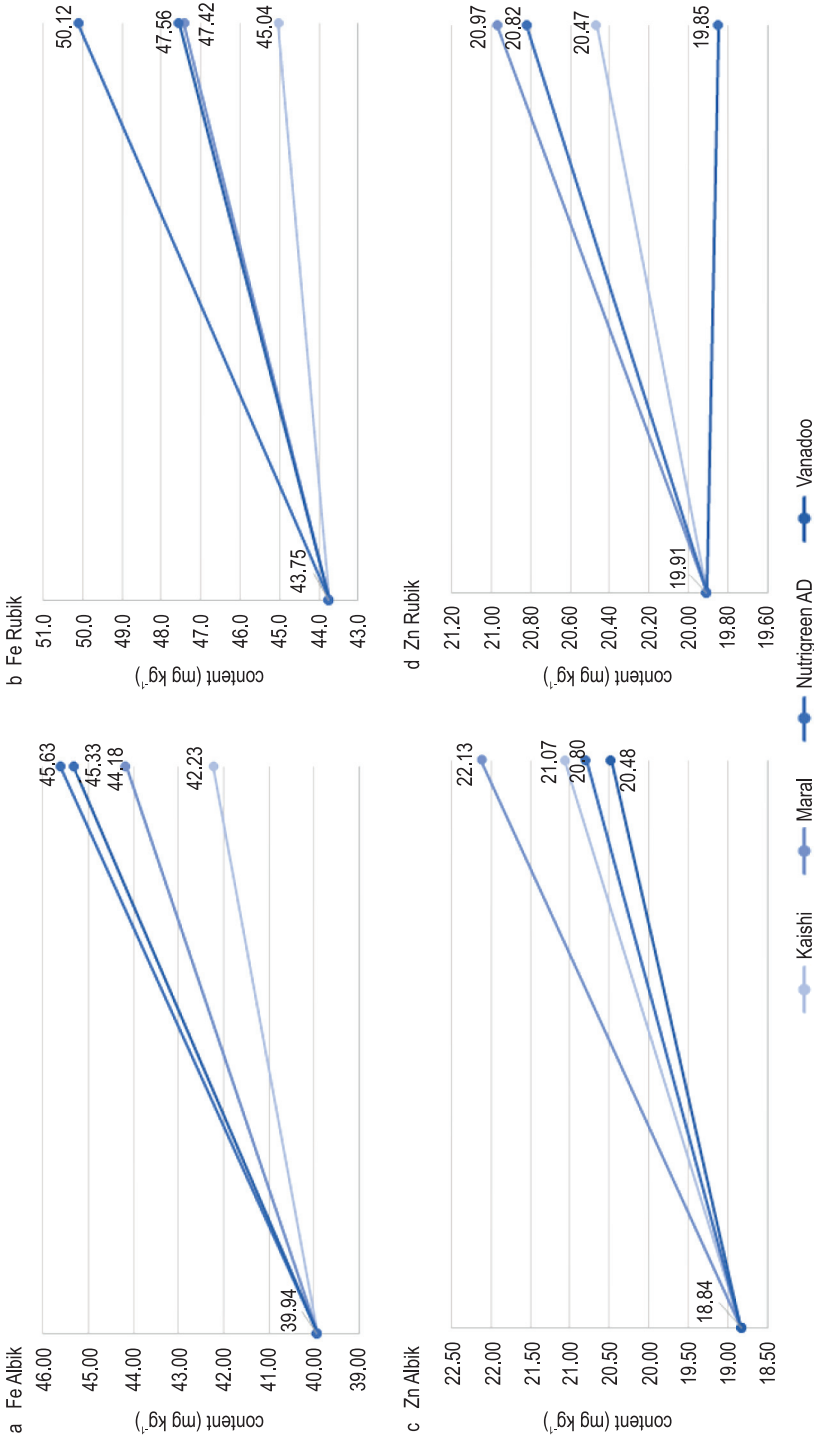


Fig. 5. The content of the micronutrients Fe and Zn after the application of biostimulants in relation to the control variant in two varieties of Jerusalem artichoke: Albaik and Rubik

in both varieties tested, but a higher increase compared to the control variant was observed in Albik (17%) than in Rubik (5%) – Figures 5c-d.

Changes in the Fe and Zn content over the three years of the experiment were also analyzed. The highest Fe content Jerusalem artichoke tubers was observed in 2021, and the lowest – in 2023, the difference being about 30% (Figure 6a). A similar trend was observed for the Fe uptake, which was about 20% lower in 2023 compared to 2021. In the case of Zn, no significant changes were observed in the content and uptake of the element (Figure 6b).

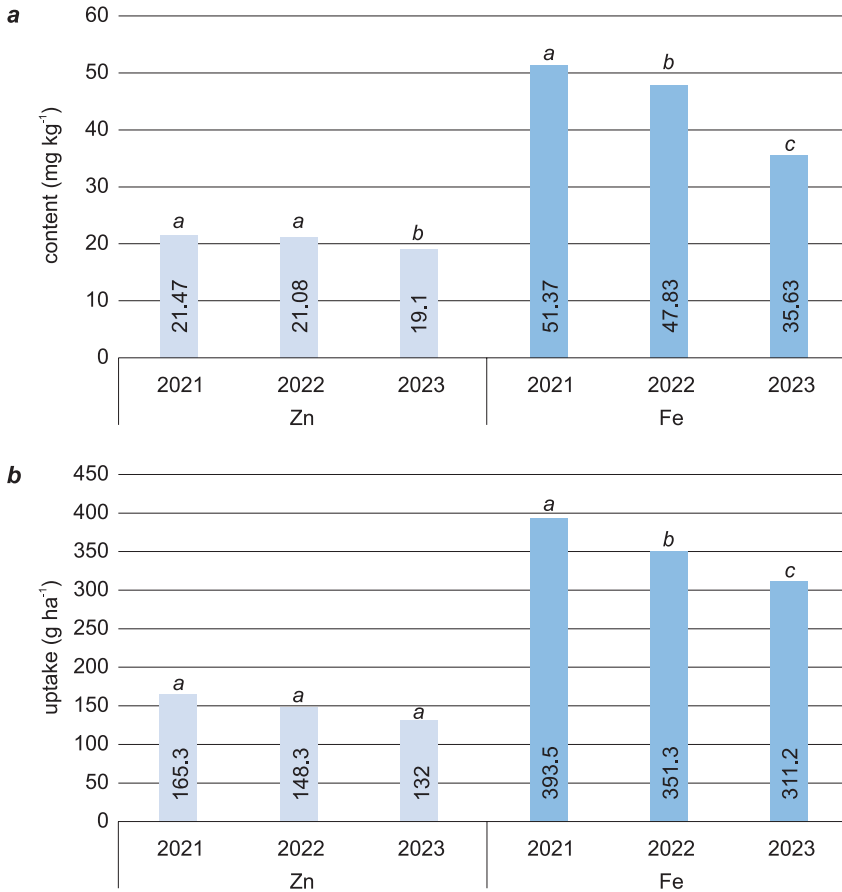


Fig. 6. Content (a) and uptake (b) of Fe and Zn in 2021-2023. Significantly different values are marked with different letters above bars (separately a-b) at $p < 0.05$

Climatic factors may also have influenced the results. Jerusalem artichoke's growth period is from May to October, but the key months are August and September, when the plant develops its tubers intensively. For the months in question, a K-factor value was calculated based on the sum of monthly precipitation and average temperature. In 2021, the K-factor

in August and September was 1.80 and 1.09, respectively (which, according to Skowera et al. (2014), is interpreted as quite humid and quite dry). In contrast, in 2023, the K-factor values in August and September indicated extremely dry weather, as they were 0.38 and 0.31., respectively. Lower temperatures can lead to slower metabolic processes in the plant, which can affect its ability to effectively take up and transform nutrients. Low rainfall leads to reduced water availability, which can limit the solubility and availability of nutrients in the soil. Reduced water availability reduces the plant's ability to take up essential minerals. Perennial stress from suboptimal conditions can lead to chronic reductions in mineral content, as plants are unable to effectively adapt to changing environmental conditions.

CONCLUSIONS

The results of the study indicate a potentially beneficial effect of the applied biostimulants. An increase in the content and uptake of Fe and Zn in tubers of *Helianthus tuberosus* L. was observed. The use of the biostimulants Kaishi, Maral, Nutrigreen AD and Vanadoo, can improve the content of essential micronutrients, but their effect on individual elements is varied. An increase in the content by about 10% over the control variant was shown after the application of Maral, Nutrigreen AD, Vanadoo for Fe and after the application of Maral for Zn. Application of any of the biostimulant increased the Fe and Zn uptake. It was found that Vanadoo had the greatest effect on the Zn uptake, while Kaishi had the weakest effect on the Fe and Zn uptake in JA tubers. Comparing two varieties of Jerusalem artichoke, Albik and Rubik, it was shown that the Rubik variety had a higher Fe content, while the Zn content was similar in both varieties.

It should be emphasized that although Jerusalem artichoke shows tolerance to various environmental conditions, changing external factors such as temperature and precipitation have a significant impact on its chemical composition and nutritional value. It should be taken into account that these factors act synergistically, affecting all aspects of the plant's physiology and its ability to effectively absorb and accumulate selected elements. During the three-year experiments, a reduction in the Fe content and uptake was observed, while Zn levels remained stable. The decrease in the Fe content in 2023 may be due to changes in temperatures and rainfall totals, which may have affected the availability of this micronutrient to plants.

The specific effects on individual elements underscores the need for further research to develop a biostimulant that could effectively increase the content of essential elements. To this end, it is also necessary to monitor climatic factors in order to optimize growing conditions and ensure high-quality yields of different varieties of Jerusalem artichoke.

Author contributions

I.M – conceptualization, writing – review & editing, funding acquisition, K.Z. – methodology, A.D. – visualization, writing - original draft preparation. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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