

ORIGINAL ARTICLE

Changes in the content of carotenoids in edible potato cultivated with the application of biostimulants and herbicide

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Abstract

An application of biostimulants is becoming an increasingly popular operation in agriculture because they positively affect crop performance and qualitative characteristics, and prevent stress-related losses. The objective of this study was to determine the effect of an application of the following biostimulants: PlonoStart, Aminoplant, and Agro-Sorb Folium, and the herbicide Avatar 293 ZC on total carotenoids in table potato tubers. The research material consisted of tubers of two table potato cultivars, Oberon and Malaga, which were produced in a 3-year field experiment. Chemical analyses of fresh material were conducted 4–6 days following harvest. Biostimulants applied with the herbicide significantly increased the table potato tuber content of carotenoids compared to tubers obtained from unamended control plots. The highest accumulation of carotenoids was determined following an application of the biostimulant Agro-Sorb Folium, and it was higher in the tubers of cv. Oberon versus cv. Malaga. It should be added that in the available literature there is no research on the effect of biostimulants on the content of carotenoids in potato, which is the main food in the world. Carotenoids perform many important functions in the human body. They exhibit antioxidant properties, which means that they protect cells from damage, and also have a beneficial effect on the immune system. Carotenoids are substances that show an indispensable effect on the health and appearance of the skin. Regular consumption of them in the form of vegetables and fruits ensures its firmness, elasticity, smoothness, as well as a healthy appearance. The protective function of carotenoids against free radicals simultaneously contributes to slowing down the aging process. This action, in turn, translates into preventing the development of cancer or diseases of the circulatory system.

Key words: bioactive compounds, growth regulators, potato cultivars, *Solanum tuberosum* L.

Introduction

Potato is one of the basic staple food plants in Europe and the world. The tubers are a valuable source of many nutrients, minerals as well as bioactive substances. Moreover, they are relatively inexpensive and available for purchase all year round (Zarzecka *et al.* 2013; Ngobese *et al.* 2017; Kazimierczak *et al.* 2019; Mishra *et al.* 2020). Depending on the cultivar, table potato tubers contain 12–18% starch which is of industrial and culinary importance. In their fresh matter, tubers have 1.7–2.9% protein which is of high nutritional

value, and is higher than any other plant protein. It contains all exogenous amino acids and is the only plant protein whose value is comparable to animal-derived protein. Potato is rich in minerals which range from 0.5 to 2% (potassium, phosphorus, calcium, magnesium, sodium, sulphur, iron, copper, manganese), and vitamins (C, B₁, B₂, B₅, B₆, H and others) (Zarzecka *et al.* 2013; Leszczyński 2012; Nassar *et al.* 2012; Sawicka *et al.* 2016). Additionally, potato tubers contain bioactive compounds including polyphenols, carotenoids,

flavonoids, anthocyanins, and vitamin C (Wegener *et al.* 2015; Keutgen *et al.* 2019; Trawczyński *et al.* 2019; Bvenura *et al.* 2022). Due to these considerations, potato tubers constitute an important source of bioactive compounds which are highly desirable diet components (Ezekiel *et al.* 2013; Bogacz-Radowska and Harasym 2016). At present, diets frequently include highly processed food which is high in sugars, saturated fats and salt, with few fruits and vegetables. An unhealthy diet is the main reason behind numerous medical conditions (Bvenura *et al.* 2022). Carotenoids are compounds which render fruits and vegetables yellow, orange and red in color. They are antioxidants and promoters of good health; they prevent or reduce an occurrence of numerous diseases, including cardiovascular diseases, a wide range of cancers, cataract and macular degeneration (Burgos *et al.* 2012; Lachman *et al.* 2016; Godwill 2018; Mishra *et al.* 2020). In nature, more than 700 carotenoids have been identified but, of these, only around 24 are present in food typically consumed by people. Carotenoids abound in spinach, broccoli, peaches, apricots, carrots, red paprika, nuts, sweet potato, and colored potato (Bvenura *et al.* 2022; Bogacz-Radowska and Harasym 2016; Zarzecka *et al.* 2022). The carotenoid content of potato tubers is affected by various factors such as genotype, agrotechnological factors, production systems, climatic conditions, storage conditions, applied plant protection agents, cooking and processing methods (Wierzbicka and Hallmann 2013; Hamouz *et al.* 2016; Ngobese *et al.* 2017; Kazimierzczak *et al.* 2019; Tatarowska *et al.* 2019; Trawczyński *et al.* 2019). In light of an increasing demand for healthy food, environmentally-friendly alternatives and means are being sought, for example, organic production or an application of biostimulants and biofertilizers which not only beneficially affect plant growth, yield, and chemical composition but also alleviate stress (Jardin 2015; Keutgen *et al.* 2019; Trawczyński *et al.* 2019). According to a UE Regulation (2019) a plant biostimulant shall be an EU fertilizing product the function of which is to stimulate plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics

of the plant or the plant rhizosphere: 1 – nutrient use efficiency, 2 – tolerance to abiotic stress, 3 – quality traits, or 4 – availability of confined nutrients in the soil or rhizosphere. Many authors have demonstrated a positive influence of biostimulants on potato plant growth and development, yield and yield structure, nutrient content, resistance to diseases and economic gain (Głosek-Sobieraj *et al.* 2019; Mystkowska and Rogóż-Matyszczyk 2019; Wadas and Dziugiel 2020; Zarzecka *et al.* 2021; Ginter *et al.* 2022; Rasheed *et al.* 2022). However, there is a paucity of literature on the effect of biostimulants on carotenoid content in *Solanum tuberosum* tubers. Accordingly, the objective of the research reported here was to assess changes in the tuber content of carotenoids following an application of biostimulants and herbicide to fields cropped to table potato. In the available literature there is no data pertaining to the effect of biostimulants on carotenoid content in potato tubers. This study hypothesized that herbicide application with the biostimulants can positively affect the carotenoid content of potato tubers.

Materials and Methods

The experimental material consisted of tubers of table potato obtained in a 3-year field experiment which was established as a two-factor split-plot arrangement with the following factors: the whole plot factors – two potato cultivars (Oberon and Malaga), the split-plot factor – three growth biostimulants and one herbicide (PlonoStart, Aminoplant, Agro-Sorb Folium; Avatar 293 ZC, respectively) as well as a distilled water-sprayed control unit. Nowacki (2021) described the test cultivars as follows: they are medium early varieties with light yellow flesh, producing high yields (Oberon and Malaga, respectively, 53.1 and 56.4 t × ha⁻¹) of large, regularly shaped tubers suitable for organic production. Foliar applications of the biostimulants were made at rates recommended by the Institute of Soil Science and Plant Cultivation in Puławy (2022). A detailed description of the application of the test products in the potato were presented in Table 1.

Table 1. Doses and methods of application of herbicide and biostimulants

Specification	Phase of the potato	Dose of the preparation [dm ³ × ha ⁻¹]
Single herbicide spraying clomazone + metribuzin (Avatar 293 ZC)	BBCH 00-08	1.5
Double spraying with the the biostimulant PlonoStart	BBCH 13-19 and BBCH 31-35	1.0 and 1.0
Double spraying with the biostimulant Aminoplant	BBCH 13-19 and BBCH 31-35	1.0 and 0.5
Double spraying with the biostimulant Agro-Sorb Folium	BBCH 13-19 and BBCH 31-35	2.0 and 2.0

BBCH – Biologische Bundesanstalt, Bundessortenamt and Chemical Industry

Single herbicide spraying clomazone and metribuzin in 2018 was done on 7 May, in 2019 on 29 April and in 2020, as in the first year of the study, on 7 May. Double spraying with each of the biostimulants in 2018 was done on 19 May (BBCH 13-19) and on 24 May (BBCH 31-35). In 2019, treatments with tested biostimulants were done on 27 May (BBCH 13-19) and on 6 June (BBCH 31-35) and in 2020, on 3 and 9 June at the same phases of the potato. The first studied biostimulant PlonoStart contains: N_{total} – 16.4%, K_2O – 0.75%, CaO – 0.07%, MnO – 0.02%, S – 941 mg $\times$ kg⁻¹, lactic acid bacteria and actinomycetes. Aminoplant biostimulant contains: N_{total} – 9.48%, N – 9.2%, N_{NH_4} – 0.88%, C_{organic} – 25%, free amino acids – 11.57% and organic matter – 87.7%. Agro-Sorb Folium biostimulant consists of N_{total} – 2.2%, B – 0.02%, Mn – 0.05%, Zn – 0.09%, total amino acids – 13.11% and free amino acids – 10.66%.

Just before potato harvest, excluding border plants, tubers of 10 randomly selected plants were dug out to take samples for further analyses. Four to 6 days after the crop plant harvest, samples of several tubers were taken, washed and the fresh material (FM) was used to conduct chemical analyses in three replicates.

Total carotenoids (TC) were determined following the protocol described in the Polish Standard PN-90/A-75101/12 (1990). Basically, the method involves extraction of carotenoids from the examined sample by means of petroleum ether followed by their determination in the obtained extract by the colorimetric method at the wavelength of $\lambda = 450$ nm. Weighed samples (10 g) of homogeneous potato mass were subjected to extraction using 150 ml alcohol-ether mixture (2 : 1). The tube contents were shaken for 10 minutes and then quantitatively transferred to a Büchner funnel to be washed until the filtrate was completely colorless. The whole amount of the filtrate was transferred into a separatory funnel and 75 ml distilled water was added to obtain layer partitioning. The lower, aqueous-alcohol layer was decanted into a conical flask and subjected to further extraction with petroleum ether, each time using 40 ml petroleum ether, whereas the ether fraction was transferred to a ground-necked flask. Extraction was terminated after all carotenoid pigments had been transferred to petroleum ether. The obtained extract was washed with distilled water. Next, it was placed in a cylinder and, after a reading of volume was taken, anhydrous sodium sulphate was added. Total carotenoids were spectrophotometrically determined in the obtained extract. In order to calculate the total carotenoid content, the following formulae were used:

$$A = 0.46605n + 0.0332 \text{ (}\mu\text{g} \times \text{ml}^{-1}\text{)},$$

where:

A – β -carotene concentration in 1ml of the examined solution ($\mu\text{g} \times \text{ml}^{-1}$),

n – β -carotene absorbance at the wavelength of 467 nm.

$$X = A \times V \times 100/G \times 100 \text{ (mg} \cdot 100 \text{ g}^{-1}\text{)},$$

where:

A – β -carotene concentration in 1ml of the examined solution ($\mu\text{g} \cdot \text{ml}^{-1}$),

V – the total volume of carotenoid extract (ml),

G – weight of sample for analysis (g).

The obtained results of chemical analyses were arranged and subjected to statistical calculations – variance analysis. Significance of differences between compared means at the significance level of $p \leq 0.05$ was checked using Tukey multiple intervals (Trętowski and Wójcik 1991). Meteorological data throughout the period from April to September in each study year, and characterization of each month are displayed in Table 2.

The average air temperature in 2018 was 3.2°C higher than the long-term mean whereas precipitation in all the growing season months (excluding September) was low. Moreover, the rainfall in September was of no account as tubers were harvested at the beginning of the month (4th September). According to Skawera *et al.* (2014), the months in 2018 were dry, very dry and relatively dry, whereas the whole growing season was dry. In turn, 2019 saw air temperatures which were higher than the long-term mean by as little as 1.2°C whereas precipitation was barely 192.6 mm and was the lowest of all the study years meaning this study year was very dry. In 2020, both the air temperature and precipitation were similar to the long-term counterparts although precipitation was irregularly distributed. The growing season was relatively dry.

Results and Discussion

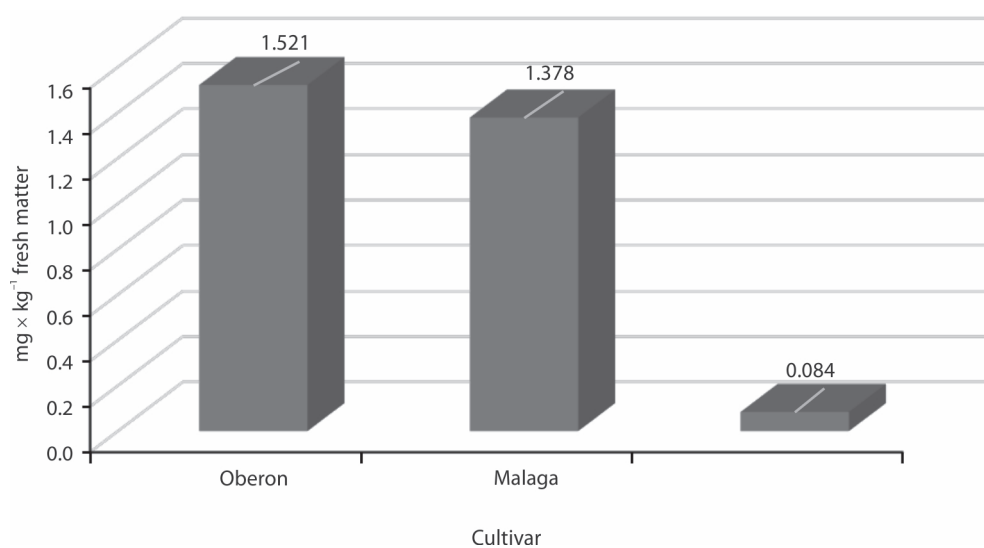
Results of the experiment demonstrated, and statistical calculations confirmed that total carotenoids (TC) in potato tubers were significantly affected by genetic traits of cultivars, applied biostimulants and herbicide as well as weather conditions prevailing during the crop's growing season (Figs. 1–3).

TC content ranged from 1.299 to 1.676 mg $\times$ kg⁻¹ fresh matter. These values are similar to those reported by other authors (Brown *et al.* 2007; Bonierbale *et al.* 2009; Murniece *et al.* 2014; Kazimierzczak *et al.* 2019). Murniece *et al.* (2014) found that carotenoid contents in white- and yellow-fleshed potatoes which were organically produced ranged from 0.89 to 3.85 mg $\cdot$ kg⁻¹ fresh matter (FM), the range being 0.68 and 3.71 mg $\cdot$ kg⁻¹ FM for conventionally grown tubers. However, the differences between the cultivation systems were insignificant. Bonierbale *et al.* (2009) found that carotenoid content may vary between 1.030 and

Table 2. Air temperature and precipitation in the years of the field experiment

Year	Month	Air temperature [°C]	Precipitation [mm]	Classification of the month*
2018	April	13.1	34.5	dry
	May	17.0	27.3	very dry
	June	18.3	31.5	very dry
	July	20.4	67.1	relatively dry
	August	20.6	54.7	dry
	September	15.9	80.6	relatively humid
	Mean/Sum	17.6	295.7	April–September dry
2019	April	9.8	5.9	extremely dry
	May	13.3	59.8	optimal
	June	17.9	35.9	very dry
	July	18.5	29.7	very dry
	August	19.0	43.9	dry
	September	14.2	17.4	very dry
	Mean/Sum	15.6	192.6	April–September very dry
2020	April	8.6	6.0	extremely dry
	May	11.7	63.5	relatively humid
	June	19.3	118.5	humid
	July	19.0	67.7	relatively dry
	August	20.2	17.9	extremely dry
	September	15.5	38.8	dry
	Mean/Sum	15.7	312.4	April–September relatively dry
Multiyear 1980–2009		14.4	307.9	

*Classification of the month was calculated according to the formula: $k = 10 P / \Sigma t$, where: P – the sum of the monthly rainfalls in mm, Σt – monthly total air temperature $>0^{\circ}\text{C}$. Ranges of values were classified as follows: up to 0.4 – extremely dry; 0.41–0.7 – very dry; 0.71–1.0 – dry; 1.01–1.3 – relatively dry; 1.31–1.6 – optimal; 1.61–2.0 – relatively humid; 2.01–2.5 – humid; 2.51–3.0 – very humid; over 3.0 – extremely humid (Skowera *et al.* 2014).

**Fig. 1.** Content of total carotenoids in cultivar potato tubers ($\text{mg} \times \text{kg}^{-1}$ fresh matter)

$21.35 \text{ mg} \times \text{kg}^{-1} \text{ FM}$. In the research by Brown *et al.* (2007) total carotenoids were within the range of 3.8 to $202.0 \text{ mg} \times \text{kg}^{-1} \text{ FM}$ and were affected by genotype.

In turn, Kazimierczak *et al.* (2019) determined lutein, the prevailing carotenoid compound in potato, whose amount was found to range from 5.8 to $11.1 \text{ mg} \times \text{kg}^{-1}$

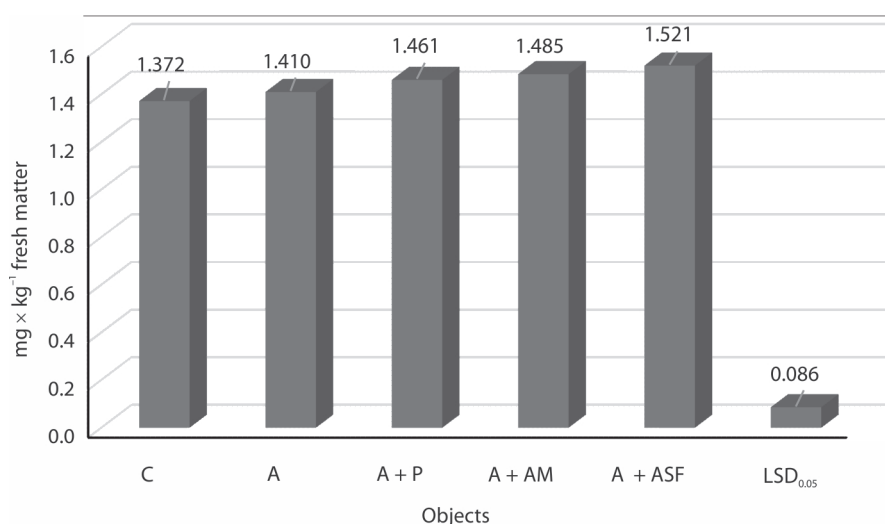


Fig. 2. Content of total carotenoids in potato tubers ($\text{mg} \times \text{kg}^{-1}$ fresh matter) depending on biostimulants and herbicide C – control object; A – Avatar 293 ZC; A + P – Avatar 293 ZC + PlonoStart A + AM – Avatar 293 ZC + Aminoplant; A + ASF – Avatar 293 ZC + Agro Sorb Folium

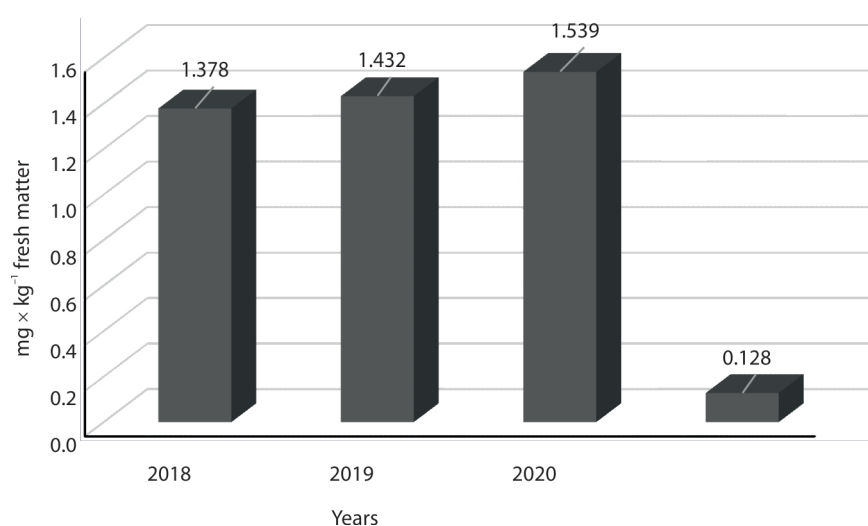


Fig. 3. Content of total carotenoids in potato tubers ($\text{mg} \times \text{kg}^{-1}$ fresh matter) depending on the year of study

FM, and was influenced by cultivar and cultivation system. The authors (Kazmierczak *et al.* 2019) recorded more lutein in conventionally versus organically grown potato tubers. In the experiment reported here, more carotenoids were accumulated by cv. Oberon than cv. Malaga (Fig. 1). Many sources mention varied carotenoid contents as affected by cultivar (Brown *et al.* 2007; Trawczyński *et al.* 2019; Lachman *et al.* 2016; Wierzbicka and Hallmann 2013). Various authors (Hejtmánková *et al.* 2013; Murniece *et al.* 2014; Hamouz *et al.* 2016) claimed that tubers with white and yellow flesh contained more total carotenoids than purple- or red-fleshed tubers.

An application in field research of the biostimulants PlonoStart, Aminoplant and Agro-Sorb Folium with the herbicide Avatar 293 ZC contributed to

a significant increase in the potato tuber content of carotenoids compared to the untreated control (Fig. 2). The most beneficial effect on total carotenoids in potato tubers was associated with an application of the biostimulant Agro-Sorb Folium and it was higher in relation to the control tubers by 10.8%. Also after the application of the other biostimulants (Aminoplant and PlonoStart) carotenoid content increased by 8.2% and 6.4%, respectively. In contrast, after application of Avatar 293 ZC herbicide only, there was a tendency to increase the TC content, but the differences were not statistically confirmed. In the literature there is no data pertaining to the effect of biostimulants on carotenoid content in potato tubers. There is only one publication of Caradonia *et al.* (2022). The authors reviewed results of published scientific studies on an application

of biostimulants in potato fields and reported their beneficial effect on production-related and qualitative characteristics mainly pertaining to the chemical composition of tubers. As far as bioactive compounds are concerned, only phenols and flavonoids have been studied whereas the effect on an accumulation of carotenoids in *Solanum tuberosum* tubers has remained unexplored so far. Wierzbicka and Hallmann (2013) found more total carotenoids and lutein in tubers harvested in plots amended with effective soil microorganisms (which contained: bacteria participating in photosynthesis, lactic acid bacteria, *Actinomyces*, yeast, and fermenting fungi) than in non-amended plots.

Grabowska *et al.* (2012) noted a significant increase in carotenoids in carrot roots following an application of the biostimulant Aminoplant at the rates of 1.5 and 3.0 dm³ × ha⁻¹. Moreover, they observed that the response of carrot to foliar application was affected by cultivar as well as environmental conditions during study years. Wszelaczyńska *et al.* (2019) demonstrated that the biostimulant Kelpak, based on a sea algae extract, increased the carotenoid content in carrot whereas Szczepanek *et al.* (2020) reported an increased concentration of bioactive compounds in the roots of *Daucus carota*. Cordeiro *et al.* (2022) used the microalga *Asterarcys quadricellulare* biomass after extracting free amino acids in two potato cultivars Clara and Cristal. Sprays of microalgae biomass increased potato tuber yields and induced biochemical changes. In the Cristal cultivar they recorded an increase in carotenoid content of 29.20 and 37.16% compared to tubers from the control, while in the other cultivar the differences were insignificant. These authors claim that an increased concentration of carotenoids can be linked to the presence of bioactive molecules such as free l-amino

acids, which stimulate plant growth. In ongoing studies our own biostimulants Aminoplant and Agro-Sorb Folium also contained free amino acids, which could initiate a significant increase in carotenoid content compared to the control object.

Weather conditions in growing seasons significantly influenced carotenoid content in table potato tubers (Fig. 3). This finding was confirmed by many authors (Grabowska *et al.* 2012; Hejtmánková *et al.* 2013; Hamouz *et al.* 2016). In the study reported here, the largest amount of carotenoids was accumulated by tubers in 2020 when precipitation was the highest and air temperatures were similar to the long-term mean – it was a relatively dry year. In the remaining study years (2018 was dry and 2019 was very dry), the content of this component was lower, being significantly lower in 2018 than 2020. Moreover, statistical analysis confirmed an interaction of biostimulant/herbicide treatments with study years, and cultivars with weather conditions, which is indicative of a considerable impact of the environment (Figs. 4–5).

Wierzbicka and Hallmann (2013) claimed that the highest total carotenoid and lutein contents are associated with optimum moisture conditions (341 mm of rainfall, similar to the long-term mean). It was the lowest in an extremely wet year. These authors confirmed an interaction between cultivars and study years as far as an accumulation of carotenoids was concerned. Trawczyński *et al.* (2019) also found that potatoes grown in a relatively moist growing season (2012) contained significantly more carotenoids in tubers than potatoes grown under wet conditions (the years 2011 and 2013). Lachman *et al.* (2016), Hamouz *et al.* (2016), Tatarowska *et al.* (2019) and Hejtmánková *et al.* (2013) reported varied carotenoid contents according

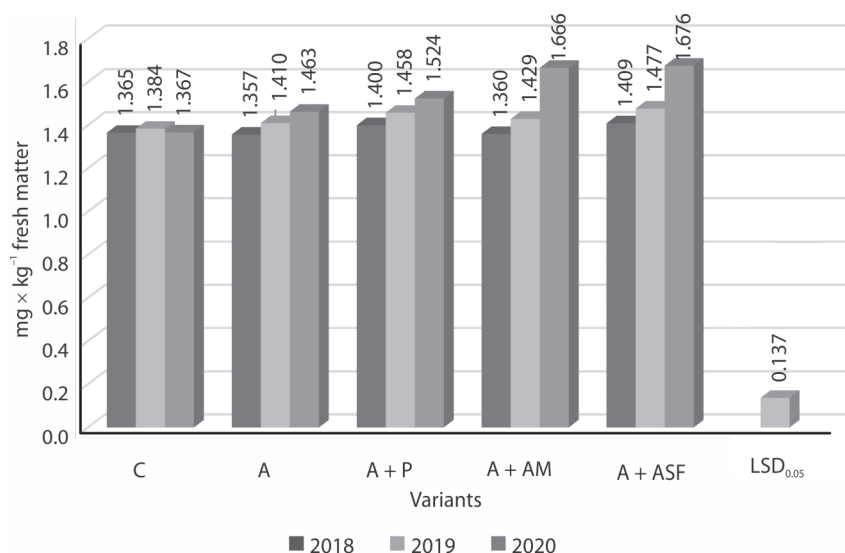


Fig. 4. Content of total carotenoids in potato tubers (mg × kg⁻¹ fresh matter) depending on the variants in the years of research (lettering as in Figure 2)

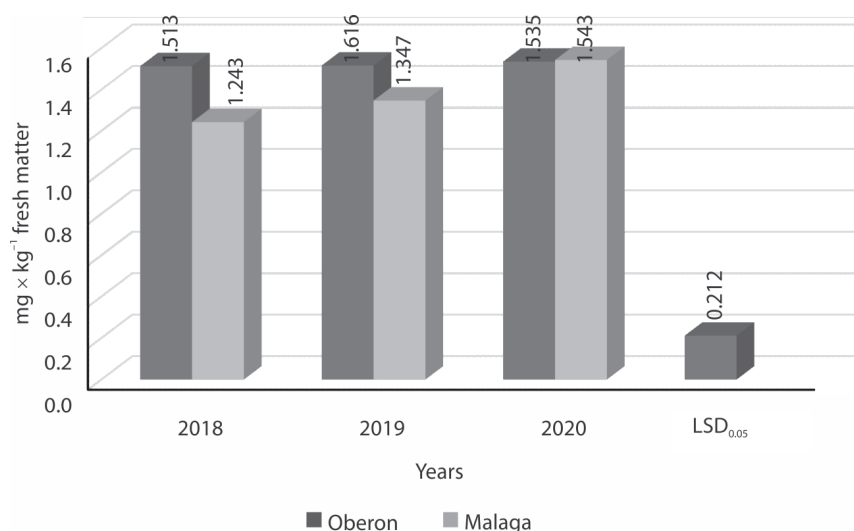


Fig. 5. Content of total carotenoids in potato tubers (mg × kg⁻¹ fresh matter) depending on the cultivar in the years of research

to cultivar, weather conditions in study years and location of the experiments.

Conclusions

Biostimulants PlonoStart, Aminoplant and Agro-Sorb Folium containing macroelements, microelements, lactic acid bacteria, actinomycetes, total amino acids and free amino acids, when applied with the herbicide Avatar 293 ZC, significantly enhanced the table potato tuber content of carotenoids compared to control tubers. An accumulation of this bioactive component was also affected by genetic traits of the test cultivars as well as weather conditions. There were confirmed interactions between biostimulant and herbicide treatments and weather conditions in study years, and cultivars and weather conditions in potato growing seasons. The most positive impact on total carotenoids in potato tubers was associated with an application of the biostimulant Agro-Sorb Folium. The observed trends indicate further research on the accumulation of bioactive compounds in potato tubers following an application of biostimulants which seem to be a promising and environmentally-friendly innovation is warranted.

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