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Nitrates content and herbicide residues in edible potato tubers under biostimulants and herbicide application conditions

Abstract. The aim of this study was to assess nitrate content and residues of the herbicide Avatar 293 ZC (clomazone and metribuzin) in table potato tubers under conditions involving an application of biostimulants and a herbicide. Tubers were sourced from a three-year field experiment. The experiment examined two factors: (I) cultivar (Oberon and Malaga) and (II) five treatments comprising herbicide and/or biostimulants: (1) untreated control (mechanical weeding only), (2) Avatar 293 ZC alone, (clomazone + metribuzin), (3) Avatar 293 ZC + PlonoStart, (4) Avatar 293 ZC + Aminoplant, and (5) Avatar 293 ZC + Agro-Sorb Folium. Nitrate content in tubers was significantly influenced by cultivar, the cultivar × year interaction, and the treatment × year interaction. However, neither the herbicide nor the biostimulants affected nitrate accumulation. Residues of Avatar 293 ZC were present only at trace levels, with clomazone and metribuzin concentrations well below the maximum residue limits (MRL).

Keywords: potato (*Solanum tuberosum* L.), nitrate accumulation, herbicide residues, biostimulants, potato cultivars

Abbreviations
ADI – acceptable daily intake
BBCH – Bundesanstalt, Bundessortenamt und Chemische Industrie
FW – fresh weight
MRL – maximum residue limits

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a basic food for people around the world and ranks fourth after wheat, rice and corn [Elrys et al. 2021, Pilana et al. 2018]. In the global food system is referred to as

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a global food security crop [Haverkort and Struik 2015, Devaux et al. 2021]. The potato is grown in about 160 countries in various latitudes. Potato yields are steadily increasing, with global production reaching 376 million tons in 2021 [Dzwonkowski 2023, FAO 2023]. Potato tubers contain valuable nutrients such as carbohydrates, high-quality protein, vitamins, dietary fiber, macronutrients and micronutrients, health-promoting compounds [Beals 2019, Giampiccoli et al. 2023, Gibson and Kurilich 2013, Zarzecka et al. 2019b]. Edible potatoes, in addition to nutrients, also contain undesirable substances called anti-nutrients. They occur naturally in the potato tubers – these are nitrates (V) and glycoalkaloids or arise as a result of agrotechnical treatments, environmental impacts, or metabolic disorders – these are pesticide residues and heavy metals. In the human body, they often hinder the absorption of nutrients, and when present in large quantities are toxic [Gorenjak et al. 2014, Urban et al. 2018, Wszelaczyńska et al. 2022, Zarzecka et al. 2010, Zarzecka et al. 2019a]. The existence of these compounds in raw materials and products of plant origin is a priority issue, as it determines the protection of human health and safety throughout the food chain [Jankowska and Łozowicka 2021, Sharma et al. 2020].

The main anti-nutrients subsyances contained in potato tubers are nitrates (V). The toxicity of these compounds to humans is low, but under the influence of gastric bacterial flora and digestive enzymes, they convert to nitrites (III), and then carcinogenic nitrosamines can be formed. Nitrites (III) also cause the conversion of hemoglobin to methemoglobin, resulting in a decrease in oxygen supply to tissues and resulting in anemia [Gorenjak and Cencič 2013, Kowalska and Kononiuk 2020, Pobereżny et al. 2015]. These nitrates are all the more dangerous because their main source in the diet is vegetables – they account for 70–90% of our intake, 32% of which comes from the consumption of potatoes and 29% from the consumption of lettuce [Gorenjak et al. 2014, Pobereżny et al. 2015] and often leads to vitamin A deficiency [Ebrahimi et al. 2020]. Standards for their content in plants and standards for daily human consumption have been established. According to Commission Regulation EU 2023/2108 of 6 October 2023 nitrate content of potatoes should not be higher than 200 mg kg^{-1} fresh tuber weight, a maximum acceptable daily intake (ADI) of nitrates for humans is 3.7 mg kg^{-1} body weight human.

Other harmful compounds are residues of plant protection products. In order to protect human health, the European Union has introduced maximum permitted levels of pesticide residues in food and feed of plant and animal origin, regulated by Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005. Hence, quantitative testing of pesticide residues in food, especially in raw materials and products consumed frequently and in significant quantities, allows estimating consumer exposure to the presence of these compounds [Kowalska et al. 2020].

The use of herbicides to reduce weed infestation is one of the basic agrotechnical measures in crop cultivation. In modern agriculture, biostimulants are often used to mitigate the adverse effects of stresses, increase the resistance of crop species, and thus have a beneficial effect on plant growth and development, as well as on the size and quality of yields [Naqve et al. 2023, Prajapati et al. 2016]. There is little information on the effect of biostimulants and herbicides on the presence of anti-nutrients in the potato. Therefore, the purpose of the conducted research was to determine the nitrate content and residue level of the herbicide (clomazone and metribuzin) in tubers of two edible potato cultivars under conditions of application of biostimulants and herbicide.

MATERIAL AND METHODS

Experiment design

The field experiment was conducted over three years (2018–2020**)** at the experimental station of the University of Siedlce, Poland, using a randomised complete block design with three replicates. The gross experimental area was 950 m^2 (60.6 m × 15.6 m) and the net area was 388.8 m^2 (12.96 m^2 × 30 plots). Each plot measured 4.8 m × 2.7 m (and contained four ridges with 48 potato plants (hand-planted at 40 cm spacing in rows 67.5 cm apart). Two medium early table potato cultivars, Oberon and Malaga, were grown (factor I, seed provided by Zamarte Potato Breeding Ltd). Five treatments (factor II) were applied: 1. control (no herbicide or biostimulants, mechanical weeding only – redlining 3 times, harrowing 1 time), 2. Avatar 293 ZC (clomazone + metribuzin) applied at BBCH 08 at a doses of 1.5 L ha^{-1}, 3. Avatar 293 ZC at BBCH 08 at a doses of 1.5 L ha^{-1} + PlonoStart applied twice (at BBCH 13–19 at a doses of 1.0 L ha^{-1} and BBCH 31–35 at a doses of 1.5 L ha^{-1}), 4. Avatar 293 ZC at BBCH 08 at a doses of 1.5 L ha^{-1} + Aminoplant applied twice (at BBCH 13–19 at a doses of 1.0 L ha^{-1} and 31–35 at a doses of 0.5 L ha^{-1}), 5. Avatar 293 ZC at BBCH 08 at a doses of 1.5 L ha^{-1} + Agro-Sorb Folium applied twice (at BBCH 13–19 at a doses of 2.0 L ha^{-1} and 31–35 at a doses of 2.0 L ha^{-1}).

The herbicide and biostimulants used in the experiment differed in their chemical composition and functional properties. The herbicide Avatar 293 ZC contained two active substances: clomazone at a concentration of 60 g L^{-1} and metribuzin at 233 g L^{-1}. Among the biostimulants, PlonoStart was characterized by the presence of lactic acid bacteria and actinomycetes, along with mineral nutrients. Its composition included total nitrogen (16.4%), potassium oxide (K_2O; 0.75%), calcium oxide (CaO; 0.07%), magnesium oxide (MgO; 0.02%), and sulfur at a concentration of 941 mg kg^{-1}. Aminoplant biostimulant was rich in organic compounds, particularly free amino acids (11.57%). It contained total nitrogen at 9.48%, including organic nitrogen (9.2%) and ammonium nitrogen ($N–NH_4$; 0.88%). Additionally, it comprised 25% organic carbon and 87.7% organic matter. The biostimulant Agro-Sorb Folium contained free amino acids at 10.66% and total amino acids at 13.11%. Its nutrient composition included total nitrogen (2.2%) as well as selected micronutrients: boron (0.02%), manganese (0.05%), and zinc (0.09%). The herbicide was applied following recommendations from the Ministry of Agriculture and Rural Development of the Republic of Poland [Ministry… 2024], and the biostimulants according to guidelines from the Institute of Soil Science and Plant Cultivation in Puławy [IUNG 2024]. The soil was a Haplic Luvisol (sandy loam texture; FAO 2015) with low to medium available phosphorus, potassium, and magnesium, and an acidic reaction (Table 1).

Table 1. Parameters of the soil before the field experiment

Parameters	Year		
	2018	2019	2020
pH KCl	5.25	5.42	5.32
Available P (mg kg^{-1})	35.2 (low)	61.0 (medium)	60.0 (medium)
Available K (mg kg^{-1})	102.1 (low)	149.0 (medium)	140.0 (medium)
Available Mg (mg kg^{-1})	36.6 (low)	61.0 (medium)	51.0 (medium)

Following the preceding crop (winter triticale), tillage followed good agricultural practice, including shallow ploughing, harrowing, and rolling [Nowacki 2020]. In autumn, before winter ploughing, manure was applied (25 t ha^{-1}), together with phosphorus and potassium fertilisation (respectively, 44.0 kg P ha^{-1} and 124.5 kg K ha^{-1}). In spring, nitrogen fertilisation was applied at 100 kg N ha^{-1}. During the growing season, three fungicide treatments and three treatments against the Colorado potato beetle were carried out. Tubers were harvested by hand from ten randomly selected plants per plot in early September. Yield components were determined immediately after harvest. Tubers >35 mm in diameter were classified as table potatoes for chemical analysis [Regulation... 2003]. Approximately 50 tubers per plot were stored at 10–12°C. After four days, samples were washed, dried with paper, and analysed fresh for nitrate content and herbicide residues.

Determination of nitrates and residues

Nitrate content was measured potentiometrically using a nitrate-selective electrode and silver chloride reference electrode [Kolbe and Müller 1987] with an Elmetron CX 721 digital measuring instrument. Calibration curves spanned 5–500 mg kg^{-1} (three replicates per series). Results were expressed as mg NO_3 kg^{-1} fresh weight (FM). Analyses followed the procedure of Wszelaczyńska et al. [2022] in the Chemical Laboratory of the Institute of Agriculture and Horticulture at the University of Siedlce. Nitrate data were analysed by two-way ANOVA, with differences between means tested at $p \leq 0.05$ using Tukey's HSD test [Trętowski and Wójcik 1991].

For herbicide residues, 8–10 tubers were collected per plot and thoroughly mixed to obtain a uniform material. The tubers were then cut into small cubes and samples of approximately 100 g were taken and homogenised. Clomazone and metribuzin were quantified using the QuEChERS (Quick Easy Cheap Effective Rugged Safe) method with LC-MS/MS, following the procedure compliant with the PN-EN 15662:2018-06 standard [2018]. The determination involved three stages: 1. extraction of the substance from the sample, 2. purification of the extract by adding a QuEChERS salt mixture, and 3. quantitative analysis. The results for clomazone and metribuzin residues were expressed in milligrams of active substance per kilogram of fresh weight (FW).

Table 2. Parameters of methods for clomazone and metribuzin

Active substance herbicide	Limits of detection (mg kg^{-1})	MRL (mg kg^{-1})
Clomazone	< 0.0001	0.01
Metribuzin	< 0.0005	0.1

The analyses were carried out in the Department of Pesticide Residue Research at the Institute of Plant Protection – National Research Institute in Poznań. The laboratory is accredited according to ISO/IEC 17025:2005 by the Polish Centre of Accreditation (PCA). The analytical method used for the determination of clomazone and metribuzin residues was characterized by limits of detection of <0.0001 mg kg^{-1} and <0.0005 mg kg^{-1}, respectively. The maximum residue limits (MRLs) for clomazone and metribuzin were 0.01 mg kg^{-1} and 0.1 mg kg^{-1}, respectively (Table 2).

METEOROLOGICAL CONDITIONS

In 2018, the mean value of the Sielianinov hydrothermal coefficient (K) for the period from April to September was 0.93, classifying the growing season as dry. April and August were classified as dry months, May and June as very dry, July as relatively dry, and September as relatively humid.

In 2019, moisture conditions were the least favourable of all years of the study. The mean hydrothermal coefficient (K) was 0.66, indicating a very dry growing season. April was classified as extremely dry; June, July, and September as very dry; August as dry; while only May was characterized by optimal moisture conditions.

In 2020, the mean value of the hydrothermal coefficient (K) was 1.05, indicating a relatively dry growing season. April and August were classified as extremely dry, May as relatively humid, June as humid, July as relatively dry, and September as humid. Despite the occurrence of drought periods in some months, moisture conditions in 2020 were more favourable than those recorded in 2018 and 2019 (Table 3).

Table 3. Hydrothermal Sielianinov index (K) in the years of the experiment

Year	Month						Mean April-–September
	April	May	June	July	August	September	
2018	0.88 dry	0.52 very dry	0.57 very dry	1.06 relatively dry	0.86 dry	1.69 Relatively humid	0.93 dry
2019	0.20 extremely dry	1.44 optimal	0.67 very dry	0.51 very dry	0.71 dry	0.41 very dry	0.66 very dry
2020	0.23 extremely dry	1.74 Relatively humid	2.05 humid	1.15 relatively dry	0.29 extremely dry	0.83 humid	1.05 relatively dry

RESULTS AND DISCUSSION

Nitrates content in potato tubers

The content of anti-nutrients in potato tubers offered to the consumer is a very important quality criterion. Parameters such as nitrates and herbicide residues have established standards for their content in individual plant species [Commission Regulation (EU) 2023 and Regulation (EC)… 2005]. The study showed that the biostimulants and herbicide used and the years of testing had no significant effect on nitrate content, while the effect of variety and interaction effects (cultivars x years and objects x years) were significant at $p \leq 0.05$ (Tables 4–5).

Table 4. Nitrates content in tubers of potato cultivars depending on weather conditions (mg kg^{-1} FM)

Cultivar	Year			Mean value	Standard deviation s
	2018	2019	2020		
Oberon	153.8^{A}	155.3^{A}	156.5^{A}	155.2^{a}	1.35
Malaga	153.0^{A}	152.1^{B}	150.9^{B}	152.0^{b}	1.05
Mean	153.4^{a}	153.7^{a}	153.7^{a}		

LSD$_{0.05}$: cultivars 1.0; years x cultivars 2.7
Lowercase letters a and b indicate significant differences between potato cultivars. Uppercase letters A and B indicate significant interactions between cultivars and years. Results marked with the same letter (a, a; A, A) indicate that the values are not significantly different.

Table 5. Nitrates content in tubers of potato depending on study objects and weather conditions (mg kg^{-1} FM)

Object	Year			Mean value	Standard deviation s
	2018	2019	2020		
Control object	151.2^{B}	151.5^{B}	155.9^{A}	152.9^{a}	2.63
Avatar 293 ZC	153.4^{A}	155.3^{A}	153.5^{B}	154.1^{a}	1.07
Avatar 293 ZC + PlonoStart	154.9^{A}	154.7^{A}	152.3^{B}	154.0^{a}	1.45
Avatar 293 ZC + Aminoplant	153.6^{A}	154.0^{A}	153.1^{B}	153.6^{a}	0.45
Avatar 293 ZC + Agro-Sorb Folium	154.1^{A}	153.1^{A}	153.5^{B}	153.6^{a}	0.23

LSD$_{0.05}$: years x objects 2.0
Lowercase letters a indicate the absence of significant differences between the study objects and between the study years. Uppercase letters A, B indicate significant interactions between the study objects and study years. Results marked with the same letters (a, a; A, A) indicate that the values are not significantly different.

Nitrates content was from 150.9 to 156.5 mg kg^{-1} fresh matter. Similar contents of these compounds are reported by other authors: Nezami and Fatemi [2024], Pobereżny et al. [2015], Zarzecka et al. [2019a]. The Oberon cv. contained significantly more nitrates than the Malaga cv. The interaction of cultivars with years showed a differential response of the cultivated creations to weather conditions. The Oberon cultivar accumulated the highest amount of these compounds in 2020, which was relatively dry, and Malaga cv. in 2018, which was dry. Variance analysis also showed that in 2018 and 2019 (dry and very dry) nitrate accumulation in tubers was higher after herbicide and herbicide with biostimulants, while in 2020 (relatively dry) the amount of these compounds decreased (Table 5). The influence of the cultivar factor is confirmed by other authors [Gorenjak et al. 2014, Wszelaczyńska et al. 2022, Zarzecka et al. 2010, 2019a]. These authors also write that the nitrate content of tubers depends on a number of factors, mainly the cultivar, climate and soil conditions and agrotechnical treatments. Naghdi et al. [(2022] found that the applied treatments had no significant effect on nitrate content, while the interaction effects of the two factors in the second year were significant, and that the amount of nitrate was at a significantly lower level than the nitrate permissible level.

Table 6*. Daily human nitrate consumption per 227 g of potato tubers under the study conditions. Average from 2018–2020 (mg kg^{-1} FM)

Object	Cultivar		Mean
	Oberon	Malaga	
Control object	35.1	34.3	34.7
Avatar 293 ZC	35.3	34.6	35.0
Avatar 293 ZC + PlonoStart	35.3	34.6	35.0
Avatar 293 ZC + Aminoplant	35.3	34.4	34.9
Avatar 293 ZC + Agro-Sorb Folium	35.2	34.5	34.9
Mean	35.2	34.5	34.9

* No significant differences ($p \leq 0.05$) were found between treatments; thus, LSD test results are not reported for this table.

Nowacki [2022] classified potato tubers according to nitrate content into three groups: low content – less than 100 mg, medium content from 100 to 200 mg and high content – above 200 mg kg^{-1}. Thus, the results obtained confirm that tubers of Oberon and Malaga cultivars accumulated an average amount of nitrates and the level of 200 mg kg^{-1} FW was not exceeded. The per capita consumption of potatoes in Poland in 2023 was 83 kg year^{-1}, that is, 227 g a day [Dzwonkowski 2023]. This did not result in exceeding the permissible daily intake of nitrates, so it does not pose a risk to human health (Table 6). ADI of nitrates for humans is 3.7 mg kg^{-1} body weight human [Commission Regulation (EU) 2023]. The test results showed that the applied treatments did not exceed the permissible daily intake of nitrates, so it does not pose a threat to human health (Table 6).

Residues content in potato tubers

No residues of clomazone or metribuzin exceeding the maximum residue limits (MRLs) established by European Union regulations were detected in any of the analysed potato tuber samples (Table 7). In most cases, residue concentrations were below the limit of detection. These findings indicate that the application of herbicides at the recommended rates and growth stages did not result in excessive accumulation of residues in potato tubers. The obtained results confirm the safety of the applied weed control treatments with respect to herbicide residues in the harvested tubers. Similar findings have been reported by other authors investigating herbicide residues in potato crops [Kumari et al. 2015]. The maximum residue limits (MRLs) for clomazone and metribuzin are presented in Table 2. In the Oberon cultivar, the presence of clomazone residues was below the detection limit (below the limit of detection). In tubers of this cultivar harvested from object 5 (Avatar 293 ZC + Agro-Sorb Folium) the presence of metribuzins was determined at 0.00078 mg kg^{-1} but these values were below the MRL. At Malaga cv. clomazone and metribuzin residues were traceable – within limits of detection but below MRL. Hu et al. [2010] after applying metribuzin and quizalofop-p-ethyl to a potato crop, and Zarzecka et al. [2010] after spraying metribuzin and flufenacet did not detect residues of these compounds. Liu et al. [2015] using thiram did not determine residues in tubers and concluded that the formulation can be used in the crop as a pesticide with low toxicity to humans and the environment. Skiba et al. [2021] used a mixture of Afalon 450 SC + Command 480 EC formulations containing linuron and clomazone and indicated the presence of clomazone, the concentration of which was 0.0002 mg kg^{-1} fresh weight of potato tubers, so it was much lower than the MRL. In the 2020 study, no residues of clomazone and metribuzin were detected, which could be due to optimal weather conditions, especially precipitation in the months of May, June and July ensuring proper plant growth and development (Table 2). Skiba et al. [2021] also found no pesticide residues in potato tubers in 2017. This was probably due to the fact that during the 2017 potato growing season there was very high precipitation, much higher than in the multi-year period, which may have contributed to the leaching of plant protection product residues.

Table 7. Occurrence of residues of Avatar 293 ZC herbicide (clomazone + metribuzin) in potato tubers in 2019*

Objects	Residues (mg kg^{-1})	
	clomazone	metribuzin
	cv. Oberon	
Control object – without herbicide	0	0
Avatar 293 ZC	not detectable	not detectable
Avatar 293 ZC + PlonoStart	not detectable	not detectable
Avatar 293 ZC + Aminoplant	not detectable	not detectable
Avatar 293 ZC + Agro-Sorb Folium	not detectable	0.00078
	cv. Malaga	
1. Control object – without herbicide	0	0
2. Avatar 293 ZC	0.00013	0.0010
3. Avatar 293 ZC + PlonoStart	0.00044	0.00032
4. Avatar 293 ZC + Aminoplant	0.00023	0.00078
5. Avatar 293 ZC + Agro-Sorb Folium	0.00012	0.00074

* Residue data are presented only for 2019, since in 2018 and 2020 no residues of clomazone or metribuzin were detected in potato tubers (all values were below the analytical limit of detection, LOD).

Currently the quality of raw materials and plant products consumed now plays an important role. The European Union has introduced a food safety strategy requiring all member states to have a program to monitor and conduct official food controls on the content of contaminants, including pesticides in accordance with the maximum levels of these compounds – MRL [Kowalska and Kowalski 2019]. Kowalska et al. [2020] analyzing 160 samples of various unprocessed raw materials and plant products, concluded that residues of labeled pesticides cannot be considered a serious threat to human health, but should be monitored.

Herbicides may influence nitrogen metabolism by modifying nitrate uptake and assimilation processes, particularly nitrate reductase activity, which can lead to increased nitrate accumulation in potato tubers. The intensity of this effect depends on environmental conditions, especially soil moisture, which affects herbicide uptake and translocation within the plant. Biostimulants may additionally modify nitrogen assimilation processes; however, their effect in the present study was inconsistent. Overall, nitrate accumulation resulted from interactions between genotype, agronomic practices, and environmental conditions regulating nitrogen metabolism.

CONCLUSIONS

1. The applied herbicide and biostimulants did not significantly affect nitrate content in potato tubers, whereas cultivar and the interactions cultivar × year and treatment × year had a significant influence on this trait.

2. Nitrate content in tubers ranged from 150.9 to 156.5 mg kg^{-1} fresh weight and did not exceed levels considered safe for human consumption. The tested cultivars were classified as having a medium nitrate content.

3. Weather conditions and genetic factors played an important role in shaping nitrate accumulation in potato tubers, as confirmed by significant differences between cultivars and growing seasons.

4. No residues of clomazone or metribuzin exceeding the maximum residue limits (MRLs) were detected in any of the analysed samples, indicating that the applied plant protection strategy ensured the safety of potato tubers.

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