

## REDUCTION OF GLYCOALKALOIDS IN POTATO UNDER THE INFLUENCE OF BIOSTIMULATORS

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**Abstract.** The aim of the conducted research was to determine the effect of biostimulators on the content of glycoalkaloids in leaves and tubers of three edible potato varieties. The experiment was established in a split-plot system in three replications. The examined factors were: I – three varieties of edible potato: Honorata, Jelly, Tajfun, and II – five types of using biostimulators: biostimulator BrunatneBio Złoto Cytokinin<sup>®</sup>, GreenOk<sup>®</sup>, Kelpak SL<sup>®</sup>, Titanit<sup>®</sup>, control variant. In the biostimulators group there were products containing functional elements, eg titanium, products based on algae extracts, and products containing amino acids. The lowest concentration of glycoalkaloids in leaves and tubers of edible potato was found in the Jelly variety – 251 mg·kg<sup>-1</sup> on average in leaves, and – 80.5 mg·kg<sup>-1</sup> in tubers, while the largest in the Tajfun variety – on average in leaves 370 mg·kg<sup>-1</sup>, in tubers 110 mg·kg<sup>-1</sup>. Under the influence of all bioregulators, there was a significant reduction in the content of harmful glycosides as compared to the control variant. The lowest concentration was recorded under the influence of the BrunatneBio Złoto biostimulator – on average in the leaves 293 mg·kg<sup>-1</sup>, in tubers 91 mg·kg<sup>-1</sup>.

**Keywords:** *Solanum tuberosum* L., glycosides, BrunatneBio Złoto Cytokinin<sup>®</sup>, GreenOk<sup>®</sup>, Kelpak SL<sup>®</sup>, Titanit<sup>®</sup>

### Introduction

Glycoalkaloids (TGA - total glycoalkaloids) are toxic steroid glycosides naturally occurring in a potato plant (*Solanum tuberosum*) (Friedman, 2006). In leaves, stems, flowers and sprouts, the content of these compounds is several times higher than in tubers. The maximum content of glycoalkaloids, regardless of the variety, is up to 200 mg·kg<sup>-1</sup> of fresh tuber weight (Mazurczyk and Lis, 2000; Barceloux, 2008). In the studies of many authors (Mazurczyk and Lis, 2000; Percival and Dixon, 1997; Lachman et al., 2001), the total content of glycoalkaloids (cTGA; content of total glycoalkaloids) in potato tubers usually ranged from 10 to 150 mg·kg<sup>-1</sup> of fresh weight. Wroniak and Mazurczyk (2006), on the basis of many years of research between 1988–2005, including studying 145 varieties showed that fluctuations in the content of glycoalkaloids constituted from 3 to 350 mg/kg of fresh tuber mass. TGA accumulation in tubers above 100 mg·kg<sup>-1</sup> of fresh mass causes significant deterioration of taste (Mazurczyk and Lis, 2000), reducing their nutritional value and may also adversely affect human health. Consumption of potatoes with excessive content of glycoalkaloids may be toxic, damaging the nervous system and cause gastrointestinal disorders (Friedman and Dao, 1992; Percival et al., 1996). According to Grenda (2003), Pusz and Płaskowska (2008), Von Bennewitz Alvarez et al. (2008), Matysiak et al. (2011) and Bulgari et al. (2015) biostimulators used in the cultivation of plants are a natural method of better utilization of plant growth conditions (temperature, air and substance humidity, amount and intensity of light) and nutrients, which in turn contributes to the increase in the quantity and quality of the crop.

Our hypothesis was that the use of biostimulators reduce the concentration of glycoalkaloids in potatoes. Here we present the impact of the types of biostimulators on

the content of anti-nutritional compounds called glycoalkaloids in leaves and tubers of three edible potato varieties.

## Materials and methods

### *The experimental site*

Field research was carried out from 2015 to 2017 in an individual farm in Międzyrzec Podlaski (51°59' N i 22°47' E), Biała Podlaska county, Poland (Figure 1).



**Figure 1.** Location of the experiment

The experiment was established in three replications using the split-plot method, on the soil included in the very good rye complex, of the IV a soil class. In individual years of research, soils differed in the content of organic matter and available macro-elements. In 2015 and 2016, the soil was characterized by slightly acidic reaction, and in the last year of research, alkaline. The content of organic matter ranged from 15.0 to 18.7 g·kg<sup>-1</sup>. The content of available phosphorus (P) was from high to very high, potassium (K) from medium to very high, and magnesium (Mg) was high. The first factor were three moderately early varieties of edible potato: Honorata, Jelly and Tajfun, and the second one, four types of biostimulators used in three dates (beginning of flowering, fully flowering and after flowering of plants):

- Control variant – without the use of biostimulators spraying with distilled water.
- Biostimulator BrunatneBio Złoto (active substances – plant hormones: auxin – 0.06 mg·l<sup>-1</sup> and cytokinin - 12 mg·l<sup>-1</sup>) at a dose of 0.20 l·ha<sup>-1</sup>.
- Biostimulator GreenOk<sup>®</sup> (active substance – humus substances 20 g·l<sup>-1</sup>) at a dose of 0.20 l·ha<sup>-1</sup>.
- Biostimulator Kelpak<sup>®</sup>SL (active substance - *Ecklonia maxima* algae extract), containing plant hormones: auxin - 11 mg·l<sup>-1</sup> and cytokinin – 0.031 mg·l<sup>-1</sup>, at a dose of 0.20 l·ha<sup>-1</sup>.
- Biostimulator Tytanit<sup>®</sup> (active substance – titanium) at a dose of 0.20 l·ha<sup>-1</sup>.

The forecrop for potato in particular years of research was winter wheat. After harvesting the forecrop, a team of post-harvest crops was made. In autumn, each year preceding planting, organic fertilization in the form of manure in the amount of 25.0 t·ha<sup>-1</sup> and mineral fertilization with phosphorus-potassium in the amount of P – 44.0 (100 P<sub>2</sub>O<sub>5</sub>·0.44) kg·ha<sup>-1</sup> (lubofos for potatoes 7%) and K – 124.5 (150 K<sub>2</sub>O·0.83) kg·ha<sup>-1</sup> (lubofos for potatoes 25%) was applied. These fertilizers were plowed pre-season plowing. Nitrogen fertilizers were applied in the spring in an amount of N 100 kg/ha (nitro-chalk 27%) and mixed with the soil using a cultivator. Potatoes were planted manually under the marker at a spacing of 67.5 x 37 cm, in the third decade of April (2015, 2016, 2017). Each plot with an area of 15 m<sup>2</sup> accounted of five ridges. Cultivation and care treatments were carried out in accordance with the requirements of correct. During the growing season, the potato plantation was protected with the following incidences: Actara 25 WG (thiametoksam) at the rate of 0.08 kg·ha<sup>-1</sup> and Calypso 480 SC (thiacloprid) at the rate of 0.1 l·ha<sup>-1</sup>, and fungicides: Copper Max New 50 WP at the rate of 2.0 kg·ha<sup>-1</sup> and Dithane Neo Tec 75 WG the rate of 2.0 kg·ha<sup>-1</sup>. Samples of potato leaves (10 leaves from central part of a stalk) for chemical analyses were taken from each treatment at the flowering stage in July (after chemical treatments). Samples of potato tubers (50 tubers) were taken from each of the plots during harvest and stored at 10-12°C, for 8-10 days. Chemical analyses were performed using fresh material from 10 representative tubers in three replications. The content of glycoalkaloids in leaves and tubers of potatoes was determined by the Bergers colorimetric method (Bergers, 1980). Fresh potatoes were homogenized; then, 150 ml of ethanol were added to a 50 g sample and extraction in a water bath was performed at 90°C. , the filtered extract was evaporated at 60°C using a rotary evaporator to the volume of 5 ml. After addition of 50 ml of 10% acetic acid and centrifuging, the liquid part was poured into a flask, the sediment was poured to the supernatant (solution above the sediment) and 4 ml of ammonia were added (NH<sub>3</sub>) to adjust to pH = 10 The flask was heated in a water bath (70°C) for 20 min, then cooled at 4°C for 3 h and centrifuged. The sediment was dissolved in 5 ml 7% phosphoric acid (H<sub>3</sub>P O<sub>4</sub>). Next, 0.2 ml of the solution were mixed with 2 ml of 85% phosphoric acid with paraformaldehyde (30 mg·l<sup>-1</sup>) and mixed again. After 40 min, absorption was recorded at the wavelength of 600 nm (solution colour changes to blue and then gets lighter). The amount of potato total glycoalkaloids was calculated based on the L-solanine standard curve. The results of the analyses are given as mg per 1 kg of fresh matter.

**Table 1.** Weather conditions during of potato vegetation [Zawady Meteorological Station, (52°03'N and 22°33'E), Poland]

| Months            | Air temperature (°C) |               |      |      | Rainfall (mm)   |              |       |       |
|-------------------|----------------------|---------------|------|------|-----------------|--------------|-------|-------|
|                   | Multi-year mean      | Monthly means |      |      | Multi-year mean | Monthly sums |       |       |
|                   | 1996-2010            | 2015          | 2016 | 2017 | 1996-2010       | 2015         | 2016  | 2017  |
| April             | 8.0                  | 8.2           | 9.1  | 6.9  | 33.6            | 30.0         | 28.7  | 59.6  |
| May               | 13.5                 | 12.3          | 15.1 | 13.9 | 58.3            | 100.2        | 54.8  | 49.5  |
| June              | 17.0                 | 16.5          | 18.4 | 17.8 | 59.6            | 43.3         | 36.9  | 57.9  |
| July              | 19.7                 | 18.7          | 19.1 | 16.9 | 57.5            | 62.6         | 35.2  | 23.6  |
| August            | 18.5                 | 21.0          | 18.0 | 18.4 | 59.9            | 11.9         | 31.7  | 54.7  |
| September         | 13.5                 | 14.5          | 14.9 | 13.9 | 42.3            | 47.1         | 13.6  | 80.1  |
| April – September | 15.0                 | 15.2          | 15.8 | 14.6 | 335.4           | 295.1        | 200.9 | 335.4 |

### Meteorological conditions

In the years of research, varied weather conditions prevailed (*Table 1*). The growing season of 2015 proved to have an average of air temperature of 15.2°C, higher by 0.2°C than the long-term mean and precipitation of 295.1 mm. The highest average air temperature was recorded in 2016 and it amounted to 15.8°C, it was higher than the long-term average by 0.8°C, while this year was characterized by the lowest precipitation – 200.9 mm, lower by 134.5 mm from the long-term sum. The highest number of rainfall was recorded in the growing season 2017–325.4 mm and the lowest average air temperature –14.6°C.

### Statistical analysis

Results of the study were analysed by ANOVA. Significance of sources of variation was checked with the Fisher-Snedecor test and the significance of differences between means was tested using the multiple comparison Tukey's test at the significance level of  $P = 0.05$ . Statistical calculations were performed in Excel using the authors' own algorithm based on the split-plot mathematical model.

### Results and discussion

The content of glycoalkaloids in potato tubers depending on the types of biostimulators used (*Table 2*). Statistical evaluations showed a significant effect of cultivars on the content of glycoalkaloids in potato tubers. The TGA content was the highest in the Tajfun variety, an average of 109.7 mg·kg<sup>-1</sup>, and the lowest in the Jelly variety, an average of 80.5 mg·kg<sup>-1</sup>. The results are similar to the results presented by Eltayeb et al. (2003), Hamouz et al. (2014) and Valcarcel et al. (2014), who stated that the genotype had a dominant effect on the content of glycoalkaloids in potato tubers. The biostimulators used in the experiment reduced the content of glycoalkaloids in tubers compared to the control. The lowest TGA concentration was noted in tubers from variants sprayed with the BrunatneBio Złoto biostimulator (variant 5), an average of 91.3 mg·kg<sup>-1</sup>. In the studies by Zarzecka et al. (2015), there was an increase in glycoalkaloids after application with herbicide and biostimulator. While Hamouz et al. (2004; 2005) and Wierzbicka (2014) observed a trend towards increased TGA accumulation in edible potato tubers.

**Table 2.** Total glycoalkaloid content in potato tubers mg·kg<sup>-1</sup> fresh matter

| Types of biostimulators<br>(variants) | Cultivars |       |        | Years  |        |       | Mean  |
|---------------------------------------|-----------|-------|--------|--------|--------|-------|-------|
|                                       | Honorata  | Jelly | Tajfun | 2015   | 2016   | 2017  |       |
| 1. Control variant                    | 85.5a     | 81.5a | 110.3a | 92.6a  | 91.2a  | 93.5b | 92.4a |
| 2. Kelpak SL                          | 84.8b     | 80.5b | 109.8b | 91.8b  | 90.4b  | 93.3b | 91.8b |
| 3. Tytanit                            | 84.9b     | 80.7b | 109.7b | 91.6bc | 90.3bc | 93.5b | 91.7b |
| 4. GreenOk                            | 84.9b     | 80.2c | 109.4c | 91.3c  | 90.0c  | 93.5b | 91.5c |
| 5. BrunatneBio Złoto                  | 84.8b     | 79.8d | 109.3c | 90.7d  | 89.4d  | 93.9a | 91.3d |
| Mean                                  | 85.0c     | 80.5b | 109.7a | 91.6b  | 90.2c  | 93.5a | 91.8  |

Explanation: cultivars – a; variants – b; years – c

There are many changes in the literature confirming the growth of TGA in tubers during the growing season due to stress conditions such as too low or too high

temperature, prolonged cold or heat conditions, lack of water or its excess, and strong sunlight (Sinden et al., 1984; Bejarano et al., 2000; Hamouz et al., 2014). The cooperation of years and used biostimulators has been proved, which indicates a different effect of the biostimulators in changing climatic conditions during the tests (Table 2). Under the influence of the BrunatneBio Złoto biostimulator in the growing season 2015-2016, the lowest TGA concentration in tubers was obtained – on average from 90.2 to 91.6 mg·kg<sup>-1</sup>, while in 2017 under the influence of Kelpak SL biostimulator, the lowest concentration of glycoalkaloids was obtained, on average 93 mg·kg<sup>-1</sup>. Weather conditions during the study years differentiated the level of glycoalkaloids in potato tubers. The least glycoalkaloids were found in 2016 with the highest average air temperature of 15.8°C and were higher than the long-term average of 0.8°C, this year characterized by the lowest precipitation – 200.9 mm, lower by 134.5 mm from the multiannual sum. The interaction of years with varieties confirmed the different accumulation of TGA in the years of research. Most TGA was found in three studied varieties in 2017, and the least in 2016 (Table 4). Similar results were described by Morris and Petermann (1985).

The content of glycoalkaloids in potato leaves depending on the types of biostimulators used (Table 3). The lowest concentration of glycoalkaloids in potato leaves was found in the Jelly variety – an average of 251 mg·kg<sup>-1</sup> and the largest in the Tajfun variety – an average of 370 mg·kg<sup>-1</sup>. The content of glycoalkaloids in potato leaves was three times higher than in tubers. Zaczeka et al. (2015) in her studies found that the content was ten times higher in leaves than tubers, while Uppal (1987) and Żołnowski (2001) showed a 40-50 times higher concentrations in leaves compared to potato tubers. Biostimulators used in the experiment reduced the glycoalkaloids content in the potato leaves as compared to the control object. The smallest concentration of these compounds in potato leaves was obtained on plots sprayed with the BrunatneBio Złoto biostimulator (average of 293.5 mg·kg<sup>-1</sup>). Other authors (Gugała et al., 2016) in relation to potato, using herbicides in combination with biostimulators (Harrier 295 ZC 2.0 l·ha<sup>-1</sup> + Kelpak SL 2.0 l·ha<sup>-1</sup> and Sencor 70WG 1.0 kg·ha<sup>-1</sup> + Asahi SL 1.0 l·ha<sup>-1</sup>) received an increased concentration of glycoalkaloids in leaves and tubers in relation to the control plot.

**Table 3.** Total glycoalkaloid contents in potato leaves mg·kg<sup>-1</sup> fresh matter

| Types of biostimulators (variants) | Cultivars |        |        | Years  |         |         | Mean   |
|------------------------------------|-----------|--------|--------|--------|---------|---------|--------|
|                                    | Honorata  | Jelly  | Tajfun | 2015   | 2016    | 2017    |        |
| 1. Control variant                 | 266.1a    | 252.6a | 371.6  | 296.7a | 293.7a  | 299.9a  | 296.8a |
| 2. Kelpak SL                       | 262.6b    | 251.2b | 369.8  | 292.0b | 292.4b  | 299.2ab | 294.5b |
| 3. Tytanit                         | 263.7c    | 250.9b | 369.3  | 292.0b | 292.1bc | 298.9bc | 294.2b |
| 4. GreenOk                         | 261.5d    | 250.2c | 369.0  | 290.8c | 291.6cd | 298.3c  | 293.6c |
| 5. BrunatneBio Złoto               | 261.2d    | 250.4c | 369.1  | 291.0c | 291.3d  | 298.2c  | 293.5c |
| Mean                               | 263.0b    | 251.1c | 369.8a | 292.2b | 292.2b  | 298.9a  | 294.5  |

Explanation: cultivars – a; variants – b; years – c

Statistical evaluations showed the cooperation of varieties with the types of biostimulators used (Table 2). As a result of the use of biostimulators in the tested varieties, a significant reduction in the content of glycoalkaloids was observed. Regardless of the biostimulators used, the Jelly variety accumulated less of these

compounds than the Honorata and Tajfun varieties. The cooperation of years and biostimulators used has been proven, which indicates different effects of the biostimulator in changing climatic conditions during the tests (*Table 2*). Under the influence of the BrunatneBio Złoto biostimulator in all growing seasons, the smallest TGA concentration was obtained – on average from 291 to 298.2 mg·kg<sup>-1</sup>. Weather conditions in the study years differentiated the level of glycoalkaloids in potato leaves. The least glycoalkaloids were found in 2015, while the most of this component was accumulated by tubers in 2017, where the highest rainfall was recorded –325.4 mm and the lowest average air temperature –14.6°C (*Table 4*).

**Table 4.** Content of glycoalkaloids in fresh mass of edible potato tubers and leaves depending on the cultivars and years of conduct of tests (mg kg<sup>-1</sup>)

| Cultivars | Potato tubers |        |        | Potato leaves |        |        |
|-----------|---------------|--------|--------|---------------|--------|--------|
|           | 2015          | 2016   | 2017   | 2015          | 2016   | 2017   |
| Honorata  | 85.0b         | 84.0b  | 86.2b  | 261.2b        | 257.8b | 269.5b |
| Jelly     | 80.8c         | 79.1c  | 81.6c  | 250.7c        | 249.2c | 253.3c |
| Tajfun    | 108.9a        | 107.5a | 112.7a | 365.6a        | 369.7a | 374.1a |

Explanation: cultivars – a; variants – b; years – c

In the present study, an interaction was observed between the varieties studied. The content of glycoalkaloids in potato leaves and tubers has a significant impact on the genetic characteristics of the variety, and weather conditions during the growing season. The use of four biostimulators contributed to the reduction in TGA in leaves and tubers of edible potato.

Summarizing, three varieties of edible potato grown in the study differed in terms of glycoalkaloid content in leaves and tubers. The lowest concentration of glycoalkaloids was found in the Jelly variety. Biostimulant BrunatneBio Gold reduced the content of glycoalkaloids in leaves and tubers of edible potato compared to the control. The weather conditions during the growing season of the potato crop can largely affect the content of glycoalkaloids in leaves and tubers of potato. The lowest content of glycoalkaloids in the leaves was found in the year 2015, and in tubers in the year 2016.

## Conclusions

The use of four biostimulators contributed to the reduction in TGA in leaves and tubers of edible potato. Summarizing, three varieties of edible potato grown in the study differed in terms of glycoalkaloid content in leaves and tubers. The lowest concentration of glycoalkaloids was found in the Jelly variety. Biostimulant BrunatneBio Gold reduced the content of glycoalkaloids in leaves and tubers of edible potato compared to the control. The weather conditions during the growing season of the potato crop can largely affect the content of glycoalkaloids in leaves and tubers of potato. The lowest content of glycoalkaloids in the leaves was found in the year 2015, and in tubers in the year 2016. In the experiment discussed in this paper, TGA levels did not exceed 200 mg·kg<sup>-1</sup> fresh weight of tubers. The three cultivars examined are thus safe for human consumption.

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