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EFFECT OF MINERAL FERTILIZATION ON HARMFUL COMPONENTS IN POTATO TUBERS WITH PURPLE-BLUE FLESH

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ABSTRACT

Background. There are few reports in the literature on the impact of agricultural technology on the content of mineral components in a type of potato, with purple-blue peel and flesh, that is not yet commonly grown in Poland. However, an increasing interest is being shown in these cultivars owing to their high content of healthy substances associated with the colour of the tuber flesh. The aim of this experiment was to determine optimal doses of soil fertilization with basic macronutrients used in potato cultivation and see how they affect the quality composition of potato tubers with purple-blue peel and flesh.
Material and methods. In the first part of the experiment (I), nitrogen was applied as urea fertilizer (46% N) at doses of 40 kg·ha^{-1}, 80 kg·ha^{-1} and 120 kg·ha^{-1}. In the second part of the experiment (II), potassium was applied as potassium sulphate (50% K) at doses of 120 kg·ha^{-1}, 150 kg·ha^{-1} and 180 kg·ha^{-1}. Statistical analysis was performed using the one-way analysis of variance (ANOVA) (Statistica 10.0, StatSoft, USA). The Fisher's LSD test was applied to assess significant differences ($P < 0.05$) between samples.
Results. The following were determined in potato tubers: content of nitrates, content of total chlorophyll pigments and level of α solanine. The results suggest that optimal doses of fertilizers applied to soil for the cv. Blue Congo potato are: nitrogen 40 kg·ha^{-1} and potassium 120 kg·ha^{-1}.
Conclusion. At this level of mineral soil fertilization, the lowest content of nitrates was observed. Also, they were optimal doses with regard to total chlorophyll and toxic α solanine.

Key words: cv. Blue Congo, fertilization, nitrates, potato, solanine

INTRODUCTION

Potato supplies around 5% of human energy demand. Consumers choose potato as a food because of its taste and colour. Apart from biologically active metabolites (polyphenols and chlorophyll), potato also contains some toxic compounds, such as glycoalkaloids, whose levels, or more precisely the underlying factors, need to be monitored (Smith *et al*., 1996; Friedman, 2006).

Potato is a typical potassium-loving crop, but it requires only half of that level of nitrogen (White *et al*., 2007). The interaction between nitrogen and potassium and their effect on yield growth and quality are indisputable (Westermann *et al*., 1994; Johnson *et al*., 2009).

Nitrogen fertilization of potato increases its content of nitrates and glycoalkaloids, while lowering the level of vitamin C (Mondy and Munshi, 1990;

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Rogozińska, 1995; Wyszkowski, 1996; Hlusek *et al.*, 2000; Mazurczyk *et al.*, 2002); in contrast, potassium fertilization raises the content of vitamin C (Kolbe *et al.*, 1995).

Under the influence of enzymes in the human digestive tract, non-toxic nitrates (V) can be transformed into nitrates (III) (Leszczyński, 2000).

Potato tubers exposed to light undergo a process of greening, due to the transformation of amyloplasts to chloroplasts. Chlorophyll is a visible and tasteless pigment and it is not harmful; meanwhile, however, toxic glycoalkaloids are being accumulated during the greening process (Anstis and Northcote, 1973; Dale *et al.*, 1992).

The accumulation of toxic glycoalkaloids, which is a cultivar-specific trait, is induced by light and some agritechnical factors (Anstis and Northcote, 1973; Dale *et al.*, 1993; Pęksa *et al.*, 2002; Wierzbicka, 2011; Leszczyński, 2012). The threshold value of glycoalkaloids above which these compounds become hazardous to people has been determined at 200 mg·kg^{-1} of fresh matter. It is rarely exceeded and a bitter taste of potato can be sensed when glycoalkaloids approximate 110 mg·kg^{-1} of fresh matter (Papathanasiou *et al.*, 1998; Leszczyński, 2012).

There are few reports in the literature on the impact of agricultural technology on the content of mineral components in a potato type, with purple--blue peel and flesh, that is not yet commonly grown in Poland. However, an increasing interest is being observed in these cultivars owing to their high content of healthy substances associated with the colour of the tuber flesh (Lachman *et al.*, 2006).

The objective of this study has been to determine optimal doses of soil fertilization with macronutrients in the cultivation of potato with purple-blue peel and flesh, represented by a less popular cultivar the Blue Congo.

MATERIAL AND METHODS

Seeds of the purple-blue potato cv. Blue Congo were purchased from the Finnish Seed Potato Centre Ltd. (Suomen Siemenperunakeskus, Finland). The field experiment was located at the Agricultural Experimental Station in Tomaszkowo (53°42’ N; 20º26’ E, Poland). Two main one-factorial trial experiments were laid out as a Latin rectangle and performed in 3 replicates, where a single factor referred to different fertilization doses of a particular element, i.e. I nitrogen and II potassium.

In the first part of the experiment (I), nitrogen was applied as urea fertilizer (46% N). The doses of nitrogen: 40 kg·ha^{-1} and 80 kg·ha^{-1} were applied to soil before potato planting, on 12 May 2015. The dose of 120 kg·ha^{-1} was split into 100 kg·ha^{-1} before potato planting on 12 May 2015 and 20 kg·ha^{-1} before the last earthing-up on 26 June 2015 (Table 1). In this part of the experiment, potassium and phosphorus were applied before potato planting (12 May 2015) at doses of 120 kg·ha^{-1} (potassium sulphate, 50% K) and 80 kg·ha^{-1} (triple superphosphate, 20% P).

Table 1. Fertilization doses of nitrogen and potassium applied to potato cv. Blue Congo

Variant	Doses, kg·ha^{-1}			
Experiment I nitrogen	0	40	80	120
Experiment II potassium	0	120	150	180

In the second part of the experiment (II), potassium was applied as potassium sulphate (50% K) at doses of 120 kg·ha^{-1}, 150 kg·ha^{-1} and 180 kg·ha^{-1}. The whole doses were applied to the soil prior to potato planting on 12 May 2015 (Table 1). In this part of the experiment, nitrogen and phosphorus were spread over the soil before potato planting (12 May 2015) at doses of 80 kg·ha^{-1} and 80 kg·ha^{-1}, respectively.

The doses of the fertilizers corresponded to the needs of cv. Blue Congo, and were applied separately because a N-K abundance in the agricultural ecosystem had already been recognised. The supplier of the purchased potato seeds had provided information about recommended nitrogen doses and concentration of nitrogen in soil. Each experimental plot had an area of 10.80 m^2 (2.25 × 4.80 m) and a harvest area of 5.85 m^2. The whole experiment covered 2.6 acres. Field experiments were carried out from May 2015 and the potatoes were collected in the last ten days of September 2015.

The experiment was established on Haplic Luvisol originating from boulder clay (average soil).

Composite soil samples were taken from each plot to a depth of 20 cm to determine the chemical properties of the soil. On the experimental sites, soil pH approximated 4.96 and the soil nutrient levels ranged from 242 mg·kg^{-1} P (the Egner-Riehm method) – very high abundance, 180 mg·kg^{-1} K (the Egner-Riehm method) – high abundance, 34 mg·kg^{-1} Mg (the Schachtschabel method) – low abundance.

The plant cultivation treatments included double earthing-up and spraying several times with insecticides. The following herbicides were used to control dicotyledonous weeds: Afalon Dyspersyjny 450 SC (*linuron*) in a dose of 2 dm^3·ha^{-1}, Sencor Liquid 600 SC (*metribuzin*) in a dose of 0.5 dm^3·ha^{-1}, Agil 100 EC (*propaquizafop*) in a dose of 1.5 dm^3·ha^{-1}. Potato blight was controlled with the systemic fungicide Ridomil Gold MZ 68 WG (*metalaxyl*-M + *mankozeb*) dosed at 2 kg·ha^{-1} and with two other preparations using' the translaminar mode of action, i.e. Acrobat MZ 69 WG (*dimetomorf* + *mancozeb*) dosed at 2 kg·ha^{-1} and Tanos 50 WG 0.7 (*cymoxanil* + *famoxat*) dosed at 2 kg·ha^{-1}. The Colorado beetle was controlled with the neonicotinoid Apacz 50 WG (*chlotianidin*) in a dose of 40 g·ha^{-1} and Calypso 480 SC (*tiachloprid*) in a dose of 0.08 dm^3·ha^{-1}.

Determination of quality characteristics

Nitrates (V) and (III)

The contents of nitrates (V) and (III), both immediately after harvest and after six months of storage, were determined by the ion-selective method (Baker and Thompson, 1992) with the application of a multi-purpose Elmetron computer device CX-721. The apparatus was equipped with a nitrate electrode, a double junction reference electrode (outer chamber filled with 0.02 M $(NH_4)_2SO_4$ solution from Merck, Germany) a specific ion meter and a pH/millivolt (mV) meter with an accuracy' of 0.1 mV. Nitrates (V) was extracted by a $KAl(SO_4)_2$ (Merck, Germany) solution and determined potentiometrically by an ion-selective electrode. This method has been approved by the Polish Centre for Accreditation. The determination limit was 30 mg·kg^{-1} and the measurement error was up to 15% (k = 2, norm.), depending on the sample matrix. Two grams of freeze-dried potato and 50 mL 1% of $KAl(SO_4)_2$ extracting solution were mixed and shaken using an IKA® KS 130 Basic orbital shaker (Germany) for 1 hour. Next, 10 mL $Al_2(SO_4)_3$ from Acros Organics, USA was added and shaken immediately before testing.

The standard solutions were made in a 0.025 M $Al_2(SO_4)_3$ background solution and de-ionised water was used in the analytical research at each stage. The content of NO_2^- ions was determined after its oxidation to NO_3^-, in a previously prepared sample of the extract according to the above method. In order to facilitate the oxidation of NO_2^- to NO_3^-, 1 ml of 30% H_2O_2 (Merck, Germany) was added and the ion-metric potential was measured after 5 minutes.

Content of chlorophyll pigments

Apparatus used –
a shaker – IKA® KS 130 Basic orbital shaker, Germany,
a centrifuge – Hettina Zentrifugen, Rotina 420 R, Germany,
1 g of lyophilisated material was transferred quantitatively into a test tube, covered with 5 ml of ethanol and vortexed for 1 min., then mixed in a shaker for 10 min, and next in a centrifuge for 15 min. at 3,500 rpm with cooling 6°. The clear supernatant is transferred to a 25 ml volumetric flask (in 3 replicates) and each flask is filled to 25 ml with alcohol. The solutions thus obtained are ready for direct determinations on a spectrophotometer at a wavelength of 653 nm for chlorophyll *a* and of 666 nm for chlorophyll *b* (SHIMADZU UV-1800, UV Spectrophotometer system Japan) (Wellburn, 1994).

Solanine

Determination of glycoalkaloid concentration

500 mg of ground lyophilized potato tissue was extracted according to Bergers (1980) and the obtained glycoalkaloids were dissolved in 0.5 mL of 7% (v/v) phosphoric acid and stored at -20°C prior to analysis. To quantify TGA content, 200 μL of extract was added to 1 mL of 0.03% (w/v) paraformaldehyde in concentrated phosphoric acid. After colour development for 20 min, the absorption at 600 nm (SHIMADZU UV-1800, UV Spectrophotometer system Japan) was measured and TGA concentration was determined based on α-solanine and α-chaconine (Sigma-Aldrich, St. Louis, MO) standard curves. The results were expressed on a dry weight basis (Abbasi *et al.*, 2016).

Statistical analysis

Statistical analysis was performed using the one-way analysis of variance (ANOVA) (Statistica 10.0, StatSoft, USA). The Fisher's LSD test was applied to assess significant differences ($P < 0.05$) between samples.

RESULTS AND DISCUSSION

Content of nitrates (V) and (III)

Potato tubers were analyzed for their content of nitrates (V) and (III) (Table 2). The applied nitrogen fertilization levels affected the accumulation of nitrates (V) and (III) in tubers.

The level of non-toxic nitrates (V) was determined. An experiment conducted by Trawczyński (2012) demonstrated an increase in the content of nitrates (V) induced by increased soil fertilization with nitrogen. The highest content of nitrates (V) he recorded was 90 mg·kg^{-1}, which was achieved at the rarely used in potato cultivation fertilization dose of 200 kg·ha^{-1}. Similar results have been reported by Jabłoński (2004), who determined increasing amounts of harmful nitrates (V), between 95.2 and 104.9 mg·kg^{-1} fresh matter, when higher nitrogen doses were applied for fertilization. Our experiment confirms the above findings regarding an increase in nitrates (V) at higher doses of mineral nitrogen fertilization, although the level of 90 mg·kg^{-1} was obtained at the dose of 80 kg·ha^{-1}, whereas the dose of 120 kg·ha^{-1} resulted in the amount of nitrate equal to 152 mg·kg^{-1}. Wszelaczyńska *et al*. (2014), who explored the influence of nitrogen fertilizer on the accumulation of nitrates (V), obtained the level of 177 mg·kg^{-1} of fresh matter in the potato cultivar Bila. Wierzbicka (2006), Wierzbicka and Lis (2002) conducted experiments, in which a level of 228-385 mg·kg^{-1} of fresh matter was determined at the nitrogen fertilization dose of 150 kg·ha^{-1}, increasing to as much as 477 mg·kg^{-1} of fresh matter when the nitrogen fertilization dose equaled 200 kg·ha^{-1}, thus far exceeding the permissible amount of 250 mg·kg^{-1}. Average potato tubers contain around 30-200 mg·kg^{-1} (Leszczyński, 2012).

Potato tubers were analyzed for the content of nitrates (III) and (V) (Table 2). The applied nitrogen fertilization levels affected the accumulation of nitrates (III) in tubers. The content of nitrates was the lowest without nitrogen nutrition, when the element most probably originated solely from the soil resources. When nitrogen fertilization was raised incrementally by subsequent 40 kg·ha^{-1} doses, the amount of nitrates peaked to 16.64 mg·kg^{-1} at the highest nitrogen fertilizer dose. The highest increase in accumulation of nitrates (III) (by 5.76 mg·kg^{-1}) was observed at a nitrogen fertilizer dose raised from 80 kg·ha^{-1} to 120 kg·ha^{-1}.

Table 2. Determination of nitrates accumulated in potato tuber in response to differentiated doses of nitrogen fertilization (experiment I)

Nitrogen doses	Nitrates (V) mg·kg^{-1}	Nitrates (III) mg·kg^{-1}
N_0	53.60 d	5.87 d
N_{40}	65.10 c	9.72 c
N_{80}	90.60 b	10.88 b
N_{120}	151.80 a	16.64 a
Mean	90.27	10.78

a, b, c, d – values marked with the same letter do not differ significantly at $P < 0.05$

Nitrates (III) is toxic to people at an amount of 1.4 mg·kg^{-1} of body mass. Nitrates (III) in potato is present in trace amounts, typically in the order of 3-5 mg·kg^{-1}, and only sporadically reach 10 mg·kg^{-1} (Leszczyński, 2000). In our experiments, a level of 17 mg·kg^{-1} was found at the nitrogen fertilization dose of 120 kg·ha^{-1}, while the average content was slightly above 10 mg·kg^{-1}.

Content of chlorophylls Chl

Experiment I demonstrated a tendency of the chlorophyll a content to increase as doses of nitrogen were raised in increments of 40 kg·ha^{-1} (Table 3).

The highest total chlorophyll content was noted at the nitrogen fertilization dose of 120 kg·ha^{-1} compared to all the other mineral fertilization levels.

The greening process coincides with the accumulation of toxic glycoalkaloids (Anstis and Northcote, 1973; Dale *et al*., 1993), which was proven in our experiment (Table 3). The highest total chlorophyll level corresponded to the highest level of solanine, in both experiment I and II (Table 3, 4) under the application of the consecutive doses of potassium fertilizer.

In experiment I, the average ratio of chlorophyll *a* to chlorophyll *b* was 0.33 (Table 3).

In experiment II, the content of chlorophyll *a*, chlorophyll *b* and total chlorophyll was the lowest at the potassium dose 120 kg·ha^{-1} (Table 4). In this experiment, the average chlorophyll *a* to chlorophyll *b* ratio was similar to that determined in experiment I, and equaled 0.34.

According to the literature, the content of chlorophyll *b* (yellow-green) is 2 to 3 times lower than that of chlorophyll a (blue-green) (Sikorski, 2001; Dżugan, 2006). In our research, a reverse relationship was detected.

According to previous studies the content of chlorophyll is correlated with the content of nitrogen and the uptake of nutrients by plants (Olfs *et al*., 2005; Szulc *et al*., 2008; Kachel-Jakubowska, 2009). This was not verified in our study.

Content of solanine

Raising the mineral nitrogen fertilization in experiment I by subsequently higher doses caused a significant increase in α solanine, up to 221.25 mg·kg^{-1} at the highest nitrogen fertilizer dose of 120 kg·ha^{-1} (Table 3).

Likewise, an increase in mineral potassium fertilization in experiment II by incrementally higher doses induced an increase in α solanine to the level of 200.57 mg·kg^{-1} at the highest dose of potassium fertilizers, i.e. 180 kg·ha^{-1} (Table 4).

The average content of α solanine in the experiment with nitrogen fertilizers was higher by 14.68 (mg·kg^{-1}) than the average value of this compound in the experiment with potassium fertilizers.

Hamouz *et al*. (2014) conducted an experiment on the content of glycoalkaloids in six cultivars with purple flesh available in Europe. The cultivar Blue Congo was notable among the six analyzed varieties by having the highest content of toxic compounds. On average, tubers of this potato contained glycoalcaloids in an amount of 58 mg·kg^{-1} of fresh matter, which was higher by 28.3 mg·kg^{-1} than that determined in the cv. Blue St. Gallery. Research has demonstrated that cultivars with purple flesh accumulate glycoalkaloids much more readily than potatoes with white or yellow flesh.

According to Anstis and Northcote, 1973; Dale *et al*., 1993; Pęksa *et al*., 2002; Wierzbicka, 2011; Leszczyński, 2012 this accumulation of glycoalkaloids is stimulated by light and by certain agritechnical factors such as fertilization. This view is supported by the results of our research.

Table 3. Determination of chlorophyll and α solanine accumulated in potato tuber in response to differentiated doses of nitrogen fertilization (experiment I)

Nitrogen doses	Chlorophyll *a* mg·kg^{-1}	Chlorophyll *b* mg·kg^{-1}	Chlorophyll *a* to *b* ratio	Total chlorophyll mg·kg^{-1}	α solanine mg·kg^{-1}
N_0	1061.0 a	3052.0 b	0.35	4113.0 a	204.80 c
N_{40}	662.0 c	3323.0 a	0.20	3986.0 b	207.17 c
N_{80}	1038.0 b	1974.0 c	0.52	3011.0 c	212.80 b
N_{120}	1063.0 a	3051.0 b	0.35	4114.0 a	221.25 a
Mean	956.0	2850.0	0.33	3806.0	211.l5

a, b, c – values marked with the same letter do not differ significantly at $P < 0.05$

Table 4. Determination of chlorophyll and α solanine accumulated in potato tuber in response to differentiated doses of potassium fertilization (experiment II)

Potassium doses	Chlorophyll *a* mg·kg^{-1}	Chlorophyll *b* mg·kg^{-1}	Chlorophyll *a* to *b* ratio	Total chlorophyll mg·kg^{-1}	α solanine mg·kg^{-1}
K_0	1061.0 a	3052.0 a	0.35	4113.0 a	204.80 a
K_{120}	836.0 b	2658.0 b	0.31	3493.0 b	187.40 c
K_{150}	1062.0 a	3050.0 a	0.35	4113.0 a	193.10 b
K_{180}	1063.0 a	3052.0 a	0.35	4115.0 a	200.57 a
Mean	1005.0	2953.0	0.34	3958.0	196.47

a, b, c – values marked with the same letter do not differ significantly at $P < 0.05$

CONCLUSIONS

Our research suggests that an optimal dose of nitrogen fertilizers applied to soil under the potato cultivar Blue Congo is 40 kg·ha^{-1}. At this level of nitrogen fertilization, the lowest content of nitrates (V) and (III) was observed as was the lowest content of solanine. The dose of 120 kg·ha^{-1} was the least preferred one as it induced the highest total chlorophyll content, which in turn generated the highest level of solanine.

Based on the research results, it seems justifiable to also claim that the lowest dose of potassium. i.e. 120 kg·ha^{-1}, was an optimal one because of the lowest total chlorophyll and α solanine it induced in the potato tubers.

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WPŁYW NAWOŻENIA MINERALNEGO NA SKŁADNIKI SZKODLIWE W BULWACH ZIEMNIAKA O FIOLETOWO-NIEBIESKIM MIĄŻSZU

Streszczenie

Niewiele jest doniesień literatury na temat wpływu agrotechniki na zawartość składników mineralnych ziemniaka o fioletowo-niebieskiej skórce i miąższu, który w Polsce nie przyjął się jeszcze w powszechnej uprawie. Obserwuje się coraz większe zainteresowanie tymi odmianami ze względu na dużą zawartość substancji prozdrowotnych związanych z wybarwieniem miąższu. Celem przeprowadzonych badań było ustalenie optymalnych dawek nawożenia doglebowego podstawowymi makroelementami w uprawie ziemniaka, wpływającymi na skład jakościowy bulw ziemniaka o fioletowo-niebieskiej skórce i miąższu. Sadzeniak fioletowo-niebieskiej odmiany ziemniaka Blue Congo zakupiono w Finnish Seed Potato Centre Ltd. (Suomen Siemenperunakeskus, Finland). Eksperyment polowy zlokalizowano w Stacji Doświadczalno--Rolniczej w Tomaszkowie (53º42’ N; 20º26’ E, Polska). Dwa doświadczenia polowe przeprowadzono metodą łacińskiego kwadratu, w trzech powtórzeniach, gdzie pojedynczy czynnik dotyczył różnych dawek nawożenia doglebowego: I – azotu, II – potasu. Wyniki opracowano statystycznie z wykorzystaniem analizy wariancji. Do oceny zróżnicowań między obiektami zastosowano test Fishera, zakładając prawdopodobieństwo błędu $P < 0{,}05$. Analizy wykonano stosując pakiet (ANOVA) (Statistica 10.0, StatSoft, USA). W pierwszym doświadczeniu (I) azot zaaplikowano w formie mocznika (46% N) w dawce 40, 80 i 120 kg·ha^{-1}. W drugim doświadczeniu (II) potas zaaplikowano w formie siarczanu potasu (50% K) w dawce 120, 150 i 180 kg·ha^{-1}. W bulwach ziemniaka określono zawartość azotanów (V) i (III), barwników chlorofilowych ogółem oraz poziom α solaniny. Z przeprowadzonych badań wynika, że optymalne dawki nawozów doglebowych dla odmiany Blue Congo to 40 kg·ha^{-1} N i 120 kg·ha^{-1} K. Na tym poziomie nawożenia obserwowano najniższy poziom azotanów (V) i (III). Były to dawki najbardziej optymalne ze względu na najmniejszą zawartość chlorofilu ogółem i toksycznej α solaniny.

Słowa kluczowe: azotany, fioletowo-niebieski miąższ, nawożenie mineralne, solanina, ziemniak