

CONTENT OF BIOGENIC ELEMENTS INDUCED BY THE INFLUENCE OF THE INOCULANT IN SELECTED CHICKPEA SLOVAK VARIETIES

Erika Zetochová¹, Alena Vollmannová², Ivana Tirdil'ová³

Address(es):

¹Erika Zetochová, National Agriculture and Food Centre - Research Institute of Plant Production, Bratislavská 122, 921 01 Piešťany, Slovak Republic, e-mail:erika.zetochova@nppc.sk

²Alena Vollmannová, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Chemistry, Tr. A. Hlinku 2, 94976 Nitra, Slovak Republic, e-mail: alena.vollmannova@uniag.sk

³Ivana Tirdil'ová, Slovak University of Agriculture in Nitra, AgroBioTech Research Center, Tr. A. Hlinku 2, 94976 Nitra, Slovak Republic, e-mail: ivana.ika.tirdilova@gmail.com

*Corresponding author: erika.zetochova@nppc.sk

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ABSTRACT

In the study, we focused on the evaluation of the content of biogenic elements induced by the addition of inoculant in six Slovak chickpea varieties. We analyzed Cu, Zn, Mn, Fe, Cr, Ni, Co, K, Na, Ca, Mg and P. We evaluated two variants from each variety control variant (A) and variant with inoculant (B). Seed inoculation was provided by the inoculant Rizobin. We used for analyzed 1 g from dry seeds from each genotype. We mineralized the samples on a MARS X-press. The result was atomic absorption spectrometry using VARIAN DUO 240FS / 240Z.

The average Cu content in A control variant was 5.95 mg.kg⁻¹ was reduced to 5.53 mg.kg⁻¹ by the addition of inoculant. For Zn, we also recorded a reduction by the addition of inoculant from 25.43 mg.kg⁻¹ of variant A to 24.95 mg.kg⁻¹ of B variant. The Mn content was the same in both variants and did not change significantly. We recorded the largest differences in the Fe content. The Fe content was reduced from 50.60 mg.kg⁻¹ variant A to 45.07 mg.kg⁻¹ variant B by adding an inoculant. The content of Cr, Ni and Co did not change significantly with the addition of inoculant. Potassium ranged from 8904.45 mg.kg⁻¹ variant A to 8720 mg.kg⁻¹ B variant. Variant A had detected a higher Na content 84.22 mg.kg⁻¹. The average content of Ca and Mg was reduced by adding the inoculant of B variant. The P content of 1400.88 mg.kg⁻¹ variant B was reduced in variant A to 1346.53 mg.kg⁻¹.

Keywords: legumes, chickpea, inoculant, biogenic elements, Slovak varieties

INTRODUCTION

Chickpeas (*Cicer arietinum* L.) is considered one of the oldest legumes and is characterized by high consumption worldwide. It is grown mainly in India, Pakistan, Iran, Ethiopia, Mexico (Raza et al., 2019). It is one of the three most cultivated crops in the world (Ghribi et al., 2015). Cultivation is widespread in more than 50 countries, Asia accounts for 90% (Kumar, 2019). It is a one-year-old pulp that is suitable for mild to dry climates, has a high heat tolerance, with sufficient soil moisture, prefers a temperate zone (Wallace et al., 2016, Bulbula, 2018).

We distinguish two types of chickpeas: Kabuli and Desi. The Kabuli type is grown in the Mediterranean. These are large seeds (100-750 mg), which are typical of a round shape, have a smooth beige surface and are grown in America. Their energy value is approximately 365 kcal / 100 g (Bulbula, 2018). Desi is grown mainly in semi-arid terrains, forming small (80-350 mg), charred seeds that have a rough and grooved surface. They are dark colored and have an energy value of 327 kcal / 100 g (Rachwat et al., 2015). Chickpeas are one of the main growing commodities among field crops (Kumar, 2019). It plays an important role in maintaining soil fertility due to biological nitrogen fixation (Cherinet and Tazebachew, 2016).

An important characteristic of legumes, where we also recommend chickpeas, is their specific property, the ability to bind atmospheric nitrogen and thus form a symbiosis of soil bacteria and rhizobia. The rhizobium consists of several families and genera (Peix, 2015).

Nodes in the roots or stems of legumes are induced by bacteria, followed by nitrogen fixation after the infection process (Peix et al., 2010). Due to this biological fixation by rhizobial strains, we could consider chickpeas to be an excellent crop and catch crop that improves soil fertility and structure. In addition, it reduces soil erosion within the agricultural production system (Shurigin et al., 2015).

Inoculation of legumes with rhizobia causes metabolic changes in plants, the most studied of which are increases in nitrogen and protein content, and which have

benefited in agriculture and improve the yield of many legumes (Morel et al., 2012). In recent years, the increased content of plant elements other than phosphorus has been studied after inoculation with phosphate-solubilizing rhizobia (Dahale et al., 2016) and at the same time the increase in potassium using K-solubilizing bacteria is currently being analyzed. (Kumar et al., 2016) Rhizobial inoculation not only increases yields, it also improves product quality by increasing the content

protein, crude fiber, fat, ash and carbohydrates in chickpeas (Aslam et al., 2010, Abdalla et al., 2013 and Singh et al., 2014).

According to Togaya et al. (2008) the rhizobia inoculation caused the increased plant height, first teat height, number of branches, number of teats per plant, number of seeds per plant and grain yield. Many studies on a global scale have shown a positive effect of rhizobia inoculation on chickpea nodulation, growth and yield (Rehan et al., 2018), which are associated with a broad root system, increased nutrient recovery (Yadav and Verma, 2014). Inoculation affects the growth and development of photosynthesis organs and the level of assimilation (Moinuddin et al., 2014), along with the synthesis of various phytohormones such as indoleacetic acid (IAA) (Verma et al., 2013).

According to Aslam et al. (2000) inoculation of chickpeas with rhizobium strains affects the number of nodules as well as the fresh weight of the root compared to uninoculated plants. We rank legume among the crops that are part of the human diet, as they are

a good and cheap source of protein, fat, carbohydrates and fiber (Bark, 1996; Embaby 2000). The minerals that chickpea plants obtain from the soil environment are arriving into seeds. (Della Penna, 1999). Chickpea seeds are thus a source of several essential minerals for humans. Content of Biogenic elements varies depending on chickpea genotype and growing conditions. (Abebe et al., 2006)

Chickpeas are characterized by protein content (18-29%) with its high digestibility (53-89%), contains carbohydrates (59-65%), fiber (3-17%), lipids (4.5-6.6%) and ash (2.48-3.50%). Compared to other legumes, it is one of the foods that lowers

cholesterol (Raza et al., 2019). We rank chickpeas in terms of nutrition as an excellent source of macronutrients, they contain minerals (phosphorus, calcium, magnesium, iron, zinc, potassium, sodium, copper and manganese) and vitamins (vitamin C, thiamine, riboflavin, niacin, pantothenic acid, folate, vitamin B6, choline, vitamin K, vitamin E and vitamin A) and β -carotene (Hoskem et al., 2017).

Copper, chromium, iron and zinc are essential micronutrients for human health. They play an important role in human metabolism and their interest is increasing in the context of information on the relationship between trace element status and oxidative diseases (Pelus et al., 1994; Fennema, 2000).

Zinc deficiency has also come to the attention of nutritionists, economists and medical scientists. Micronutrient malnutrition appears to be a serious global threat, affecting more than 33% of the world's population (WHO 2012). The global zinc deficiency affects about 1.1 billion people, where pregnant women and children are most at risk. Zinc deficiency results in health disorders such as immune system abnormalities, impaired physical growth and learning, and an increased risk of infections (Gibson 2006; Prasad 2007).

Magnesium, which is an important part of the basic processes of energy production and nucleic acid synthesis, is part of more than 300 enzyme systems (Saris et al. 2000). It is also involved in the regulation of muscle contraction (including cardiac), blood pressure and insulin metabolism, as well as in the synthesis of DNA, RNA and proteins (Gröber et al., 2015).

Sodium is a very important mineral for human health. It maintains the volume of plasma, regulates the water content in the body and ensures the balance of electrolytes. In addition, it is responsible for nerve impulse transmission and normal cell function. Increased sodium in a person's diet can cause high blood pressure. This problem is usually associated with high consumption of sugar and fat, in addition to salt (Elias et al., 2020).

Phosphorus is a basic element of hydroxyapatite, a key inorganic component of bone. It is also essential for many cellular compounds, such as phospholipids, phosphoproteins, nucleic acids, and adenosine triphosphate (ATP) (Arnaud and Sanchez, 1996).

Lipids (4–10%) are also present in small concentrations in chickpeas, unsaturated fatty acids are also represented, especially linoleic (54.7–56.2%), oleic (21.6–22.2%), linolenic (0.5–0.9%), palmitic (18.9–20.4%) and stearic (1.3–1.7%) (Rachwat et al., 2015). In addition, alkaloids, lectins, saponins, phytic acid and trypsin, chymotrypsin and α -amylases are present. (Rachwat et al., 2015, Chen et al., 2014). Finally, chickpeas contain phytochemicals such as phenols, which represent 0.72 to 1.81 mg / g of seed (Rachwat et al., 2015). The study was aimed on content of biogenic elements which were induced by the influence of inoculant on the content of biogenic elements in selected chickpea Slovak varieties.

MATERIAL AND METHODS

Material for analysis

The plant material for analyses was sown on the field trials plots at the National Agricultural and Food Center - Research Institute of Plant Production in Piešťany (GPS coordinates 48.5917973 and 7.827155). Due to their location, the climatic conditions of Piešťany are very favourable. Piešťany is one of the warmest areas in Slovakia. Their climate is typically lowland, slightly dry, but also slightly windy. The average annual air temperature is 9.4 °C. The annual average precipitation is 611 mm. Due to favourable climatic conditions and soil fertility was in the past this area considered a major agricultural regions of Slovakia. In our work we analysed 6 selected genotypes of chickpea (*Cicer arietinum* L.) (Table 1). All selected genotypes are of Slovak origin and were provided for this study by the Gene Bank of the Slovak Republic.

Two variants were seeded from each genotype, a control variant (A) and an inoculant variant (B). The size of individual plots for a plant material was 5.2 x 1.5 m. Seed inoculation was provided by the inoculant Rizobin, which is used to inoculate legume seeds. It is manufactured in England (Legume technology Ltd). It has a high content of live bacteria (5 * 10⁹). An organic polymer was used as a binder in the preparation. Rizobin was mixed to the seeds by manual application at a dose of 350 g. ha⁻¹. We evaluated the content of biogenic elements from measured values in 2019. Samples of selected genotypes of chickpea were taken at full maturity, dried and subsequently purified. Each analysis was done using 1 g of average sample in four repetitions.

Table 1 Chickpea (*Cicer arietinum* L.) accession analysed.

Chickpea / Genotypes	Origin
Krajova z Kralovej	SVK
Maskovsky Bagovec	SVK
Businsky	SVK
Slovak	SVK
Beta	SVK
Alfa	SVK

Determination of biogenic elements

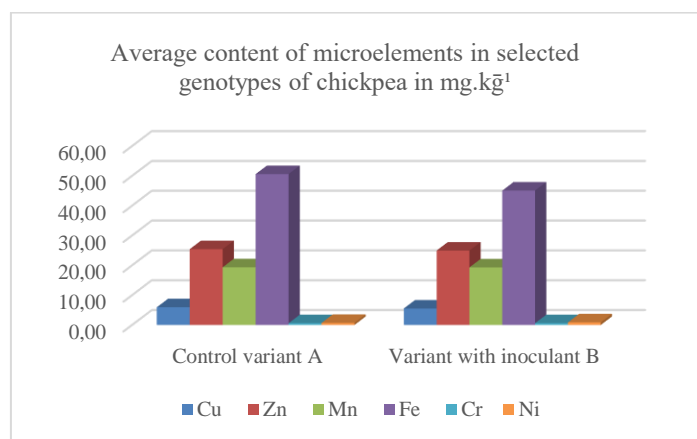
We used dry seeds, 1 g from each genotype, to determine the content of biogenic elements. Sample analyses were performed in two steps. In the first step, we mineralized the sample with the addition of 10 cm³ of oxidized HNO₃. Mineralization of the samples was performed by microwave digestion in a MARS X-press. After mineralization, in the second step, the mineralizes were filtered and filled with distilled water into a 50 ml volumetric flask. Atomic absorption spectrometry using a VARIAN DUO 240FS / 240Z instrument was used to determine the content of biogenic elements in the plant material. In order to determine the safety of plant raw materials as objectively as possible, all determined values were also recalculated to the dry matter content in mg.kg⁻¹.

Statistical methods

Each parameter was tested in four repetitions, using standard statistical methods. The statistical program STATGRAPHICS Centurion XVI, 2009 from StatPoint Technologies, Inc www.STATGRAPHICS.com was used. The multifactor ANOVA method was used to evaluate the influence of chickpea varieties on the examined parameters. In addition, we used the comparison of two independent variants by t-test to compare means, the F-test to compare standard deviations, the Mann-Whitney (Wilcoxon) W-test to compare medians, and the Kolmogorov-Smirnov test to compare the distributions of the two samples.

RESULTS AND DISCUSSION

In our study, we focused on the content of biogenic elements induced by the addition of inoculant in dried chickpea seeds. From the micro elements we evaluated the content of Cu, Zn, Mn, Fe, Cr, Ni and Co. Comparison of average values of microelement content in selected chickpea genotypes in control A variant and the variant with the addition of inoculant are given in Graph 1.



Graph 1 Comparison of average values of microelement content in selected chickpea (*Cicer arietinum* L.) genotypes in both variants A and B in mg.kg⁻¹.

The copper content in control A variant ranged from 5.40 mg.kg⁻¹ (Slovak) to 6.60 mg.kg⁻¹ (Businsky). The addition of inoculant B variant increased the copper content by 3.45% (Alfa), 1.85% (Slovak). In other varieties the copper content decreased by adding the inoculant (Table 2,3). Based on a statistical comparison of the copper content in A and B variants we found, that since the P-value is less than 0,05, from t-test, W-test and Test KS, there is a statistically significant difference between the averages of these two samples at the 95.0% confidence level (Table 4). Copper is present in several enzymes. Some of them are very important for Fe metabolism. Deficiencies of Cu are infrequent.

According to Özcan et al. (2013) measured in seeds Zn contents of 31.32 mg.kg⁻¹ (*C. arietinum* L.). Acceptable Zn intake will ensure normal reproduction and functioning organism (Hotz and Brown 2004). The highest zinc content in control variant A was recorded in the variety Bušinsky 26.20 mg.kg⁻¹, the variety Beta 24.70 mg.kg⁻¹ contained the least Zn. By adding the inoculant, the zinc content was lower in all varieties except the variety Slovak, where the zinc content was higher by 3.47% (Table 2,3).

Manganese (Mn) is an important component of the synthesis and activation of several enzymes. It is also involved in the regulation of glucose and lipid metabolism in humans. (Li and Yang, 2018). We measured the manganese content from 17.10 mg.kg⁻¹ (Beta) to 21.10 mg.kg⁻¹ (Businsky). The highest increase by the addition of inoculant was found in the variety Maskovsky Bagovec (7.85%).

We found that the content of Zn and Mn in the monitored samples in A and B variants, based on a statistical comparison from the F-test, the value of P is less than 0.05, there is a statistically significant difference between the averages of these two samples at a confidence level of 95.0%. (Table 3). By statistical evaluation based on the comparison of two samples using t-test, W-test and K-S test, there is no statistically significant difference between the averages of these two samples at the level of 95.0%. confidence level (Table 3).

Legumes generally have a high iron and mineral content (Sundberg, 2002). Fe concentrations in chickpeas have been found to range from 3 to 14.3 ppm (Wood and Grusak, 2007), but due to the presence of naturally occurring inhibitors, only a small amount is bioavailable (Hemalatha et al., 2007). In our samples the iron content ranged from 44.50 mg.kg⁻¹ (Beta) to 58.90 mg.kg⁻¹ (Businsky) in A variant. Due to the addition of the inoculant, all varieties had a reduced Fe about content of 1.80% to 21.22% (Table 2.3). Statistical comparison of the Fe content in A and B variants we found, that since the P-value is less than 0,05 in all tests, there is a statistically significant difference between the averages of these two samples at the 95.0% confidence level (Table 4). The obtained parameters of our analysis are comparable with Wang and Daun (2004), who reported a range of 2.50–5.20, 0.40–0.90 and 4.30–7.90 mg/100 g for Zn, Cu, and Fe, respectively. The Beta variety had the same chromium content in both variants of 0.70 mg.kg⁻¹. In variant A, the chromium content ranged from 0.40 mg.kg⁻¹ (Krajova z Kralovej, Maskovsky Bagovec) to 0.80 mg.kg⁻¹ (Businsky). The chromium content was increased by 50% by adding an inoculant in the Maskovsky Bagovec variety (Table 2,3). By statistical evaluation of the Cr content in A and B variants based on the comparison of two samples using t-test, W-test, F-test and K-S test, there is no statistically significant difference between the averages of these two samples at the level of 95.0% confidence level (Table 4). The nickel content was from 0.50 mg.kg⁻¹ (Krajova z Kralovej, Maskovsky Bagovec) to 1.00 mg.kg⁻¹ (Businsky). The nickel content was reduced by 50% (Beta) by the inoculant addition in B variant. In other varieties, the nickel content was increased by the addition of an inoculant. Krajova z Kralovej and Businsky had the same cobalt content in both variants 0.40 mg.kg⁻¹. The increased cobalt content in all monitored varieties (Table 2.3) was recorded by the addition of inoculant.

Table 2 Average values of microelement content in selected chickpea (*Cicer arietinum* L.) genotypes in control A variant.

Crop	Content of selected microelements in chickpea (<i>Cicer arietinum</i> L.) in control A variant/mg.kg ⁻¹						
	Cu	Zn	Mn	Fe	Cr	Ni	Co
Chickpea varieties							
Krajova z Kralovej	5.90	25.10	19.70	51.60	0.40	0.50	0.40
Maskovsky Bagovec	6.10	25.00	19.10	51.80	0.40	0.50	0.20
Businsky	6.60	26.20	21.10	58.90	0.80	1.00	0.40
Slovak	5.40	25.90	19.40	51.10	0.60	0.80	0.30
Beta	5.90	24.70	17.10	44.50	0.70	0.80	0.20
Alfa	5.80	25.70	19.70	45.70	0.70	0.60	0.40

Table 3 Measured average values of microelement content in selected chickpea (*Cicer arietinum* L.) genotypes in variant with addition inoculant B.

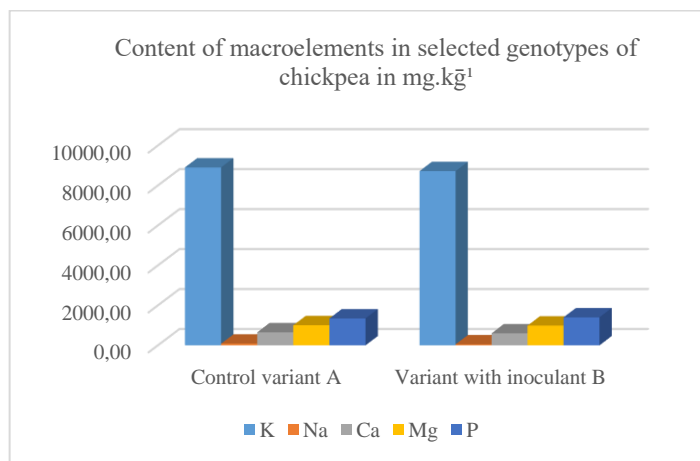
Crop	Content of selected microelements in chickpea (<i>Cicer arietinum</i> L.) in B variant mg.kg ⁻¹						
	Cu	Zn	Mn	Fe	Cr	Ni	Co
Chickpea varieties							
Krajova z Kralovej	5.20	23.80	18.70	44.10	0.50	0.80	0.40
Maskovsky Bagovec	5.80	24.90	20.60	45.20	0.60	0.90	0.30
Businsky	5.90	25.50	19.70	46.40	0.70	1.20	0.40
Slovak	5.50	26.80	18.50	48.80	0.40	1.00	0.40
Beta	4.80	22.40	17.40	43.70	0.70	0.40	0.30
Alfa	6.00	26.30	21.00	42.20	0.80	1.00	0.70

Table 4 Measured values of microelement content in selected chickpea (*Cicer arietinum* L.) varieties in the monitored variants A and B with P-values derived from t-test, F-test, W-test and K-S Test.

Element	Chickpea A (average ± SD)	Chickpea B (average ± SD)	t ¹ -test (P-value)	F ² -test (P-value)	W ³ -test (P-value)	K-S ⁴ -test (P-value)
Cu	5.950 ± 0.394	5.533 ± 0.463	0.001	0.469	0.004	0.031
Zn	25.433 ± 0.585	24.95 ± 1.636	0.189	0.012	0.397	0.259
Mn	19.350 ± 1.229	18.630± 3.313	0.381	0.000	0.649	0.259
Fe	50.600 ± 5.154	45.067 ± 2.166	0.000	0.000	0.000	0.000
Cr	0.6001 ± 0.167	0.616 ± 0.147	0.719	0.502	0.812	0.893
Ni	0.700 ± 0.200	0.884 ± 0.271	0.009	0.320	0.005	0.005
Co	0.333 ± 0.098	0.418 ± 0.147	0.014	0.009	0.057	0.259

¹ t-test to compare means; ² F-test to compare standard deviations; ³ Mann-Whitney (Wilcoxon) W-test to compare medians; ⁴ Kolmogorov-Smirnov test to compare the distributions of the two samples.

Minerals from the soil environment are transported to chickpea plants and get into the seeds (Grusak a DellaPenna, 1999). The main minerals that a plant provides to humans (e.g., Na, I, Se and Cr), may not be important to plants. Many of these essential minerals provide chickpea seeds to humans. From macro elements we focused in our experiment on content of K, Na, Ca, Mg and P in selected varieties of chickpea (Graph 2.) in control A variant and variant with inoculant B.



Graf 2 Comparison of average values of macroelement content in selected chickpea (*Cicer arietinum* L.) genotypes in both variants A and B in mg.kg⁻¹.

The major and most abundant cation in the intracellular fluid is potassium. It plays an important role in maintaining cell function. Its value determines through the cell

membrane the potential of the cell membrane, which is ensured by the ubiquitous ion channel Na-K (Na + -K +) ATPase pump (Stone et al., 2016). Potassium content varied from 8198.70 mg.kg⁻¹ (Slovak) to 9221.20 mg.kg⁻¹ (Alfa) in control A variant. The addition of inoculant in B variant reduced the content of potassium in all varieties except Beta, where the content of potassium was 0.45% higher.

Another evaluated element was calcium. The most common element in the body is calcium, which is very important and necessary for many functions in the human body. Up to 99% of Ca occurs in bones and teeth. Ca metabolism also contains proteins, vitamin D and P (Beto, 2015). Calcium is most contained in seed cover. In the case of Ca deficiency, it would be appropriate to consume whole chickpea seeds (Abebe et al., 2006). Özcan et al. (2013) report Ca values of 1.309 mg.kg⁻¹ (*C. arietinum* L.) The content of calcium in control A variant ranged from 559.60 mg.kg⁻¹ (Beta) to 773.80 mg.kg⁻¹ (Slovak). In variety Slovak, the Ca content decreased by 26.10% due to the inoculant.

Of the macroelements, chickpeas are a rich source of phosphorus and magnesium. The lowest magnesium content was found in the variety Krajova z Kralovej 949.10 mg.kg⁻¹, the highest in the variety Slovak 1061.00 mg.kg⁻¹ in control A variant. The addition of inoculant reduced the magnesium content in all varieties from 0.60% (Beta) to 12.58% (Slovak). A statistically significant difference between chickpea A and chickpea B was proven for potassium (W-test, K-test), magnesium (W-test), and calcium (t-test, W-test) P<0,05.

The lowest sodium content in the Alfa variety was 68.80 mg.kg⁻¹, the highest in the Maskovsky Bagovec variety was 103.20 mg.kg⁻¹ in control A variant. The effect of inoculant in B variant reduced the sodium content in all varieties monitored.

The last element monitored was phosphorus. Its content in control A variant ranged from 1155.10 mg.kg⁻¹ (Slovak) to 1522.10 mg.kg⁻¹ (Maskovsky Bagovec). Addition of inoculant increased the content by 16.33% (Businsky). It has an important position in human structure and metabolism. By statistical evaluation of

the P content in A and B variants based on the comparison of two samples using t-test, W-test, F-test and K-S test, there is no statistically significant difference

between the averages of these two samples at the level of 95.0% confidence level (Table 5).

Table 5 Measured values of monitored macro-elements in selected chickpea (*Cicer arietinum* L.) varieties in the monitored variants A and B with P-values derived from t-test, F-test, W-test and K-S Test.

Element	Chickpea A (average ± SD)	Chickpea B (average ± SD)	t-test (P-value)	F-test (P-value)	W-test (P-value)	K-S test (P-value)
K	8904.450 ± 341.971	8720.00 ± 308.144	0.0557	0.621	0.003	0.004
Na	84.216 ± 12.025	50.217 ± 9.125	0.000	0.193	0.000	0.000
Ca	651.233 ± 77.962	601.883 ± 58.499	0.017	0.176	0.021	0.139
Mg	1007.180 ± 43.059	990.400 ± 46.643	0.202	0.704	0.049	0.139
P	1346.530 ± 135.657	1400.880 ± 171.465	0.229	0.268	0.268	0.005

¹ t-test to compare means; ² F-test to compare standard deviations; ³ Mann-Whitney (Wilcoxon) W-test to compare medians; ⁴ Kolmogorov-Smirnov test to compare the distributions of the two samples.

CONCLUSION

In our study, we focused on the effect of inoculation on the content of biogenic elements in the seeds of selected Slovak chickpea genotypes. We evaluated Cu, Zn, Mn, Fe, Cr, Ni and Co from micro elements and K, Na, Ca, Mg and P from macro elements. Kaya et al., (2018) are reported the content of macro elements K from 9811 to 14 370 ppm, Ca from 886 to 3008 ppm, Mg from 1218 to 2037 ppm, Na from 10 to 507 ppm and P from 3109 to 5503 ppm in the selected Turkey chickpea without influence of inoculant. Soil suitability of individual cultivars, soil conditions, fertilization, irrigation and weather etc. are among the factors affecting the elemental contents of plants (Kan et al., 2005). According to Zia-Ul-Haq et al. (2012) in the evaluated legumes of Pakistan (Desi chickpeas, Kabuli chickpeas, lentils, mung beans, mashed beans and peas), were detectable amounts of 15 to 31 g.kg⁻¹ Na, 189 to 210 g.kg⁻¹ Ca, 152 to 166 g.kg⁻¹ P, 169 to 196 g.kg⁻¹ K, 135 to 167 g.kg⁻¹ Mg, 6 to 10 mg.kg⁻¹ Fe, 11 to 17 mg.kg⁻¹ Cu and 24 to 39 mg.kg⁻¹ Zn. According to literature sources, some differences in mineral content have been demonstrated. The differences found can be influenced by different legumes, the location, the harvest period and the nutritional status of the plants and many other factors that affect the mineral content.

We found that inoculation did not significantly affect the contents of individual elements. A statistically significant difference was reflected in the content of Fe on the comparison of two samples using t-test, W-test, F-test and K-S test. Conversely, statistical evaluation of the P content in variants A and B based on the comparison of two samples using t-test, W-test, F-test and KS test is not a statistically significant difference between the averages of these two samples. at a confidence level of 95.0%. Statistical evaluation of the Cr content in variants A and B based on the comparison of two samples using t-test, W-test, F-test and KS test, we found that there is no demonstrable difference between the averages of these two samples at the level of 95.0% level of confidence.

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