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Mineral composition of chickpea grain (*Cicer arietinum* L.) depending on the fertilization with liquid organic fertilizers

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The objective of the study is to establish the effect of the liquid organic fertilizers Naturamin Plus and Amalgerol Essence on the mineral composition of chickpea grain (*Cicer arietinum* L.). The experiment was conducted in the period 2019-2021 in the region of Central South Bulgaria. Fertilizers were tested in two phases of development of the chickpea: growth phase (4^{th} leaf) and beginning of flowering. The results obtained about the mineral composition were processed with ANOVA. The application of liquid organic fertilizers in chickpea has a proven positive effect on the N content in the grain. The highest content was found after treatment with Amalgerol Essence at a dose of 3.0 l/ha at the beginning of the flowering phase. The liquid organic fertilizers Naturamin Plus and Amalgerol Essence do not significantly change neither the content of the other macroelements (P, K, Ca, Mg) nor the content of the trace elements Fe, Cu and Mn in chickpea grain. Treatment of chickpea with Naturamin Plus and Amalgerol Essence at the beginning of the flowering phase results in reduced Zn content in the grain. During fertilization with Naturamin Plus and Amalgerol Essence a tendency to increase the Ca:P ratio in both phases of application was observed. The fertilizers had a more pronounced effect on the Ca:Mg ratio when applied at the beginning of the flowering phase.

Key words: chickpea, liquid organic fertilizers, nitrogen content, mineral composition, Ca:P ratio, Ca:Mg ratio

INTRODUCTION

Chickpea is the third-largest grain bean culture in the world beans production. Traditionally it is grown in Asia, Europe, Africa and Australia as a source of cheap protein for human nutrition [1]. It not only contributes to solving the protein problem in feeding people, but also supplies essential vitamins and minerals [2]. About 100 g of chickpea grains can meet the daily dietary needs of Fe and Zn and 200 g can meet the needs of Mg. Chickpea consumption has a beneficial effect on some diseases such as CVD, type 2 diabetes, digestive diseases and some cancers and is an alternative for the prevention of chronic degenerative diseases [3−5].

Chickpea can be used as a high-energy and high-protein food in animal diets to assist milk, meat and/or egg production [6]. Chickpea grain is a good source of protein in feeding broiler chickens and at the same time it has a beneficial effect on the dietary value of chicken meat and the expected enhancing effect on consumer health [7]. Mineral substances are responsible for the functioning of important enzyme systems in the human, animal and plant organism [8]. The most common mineral in the human and animal body is Ca – structural component of bones and teeth, important for the normal flow of many vital functions. Ca deficiency results in rickets, slow growth in animal development and osteoporosis, rickets and other human diseases [9-11]. Phosphorus is often discussed in connection with Ca, as the two minerals function together in bone formation [10]. Ruminants can tolerate large variations in the Ca:P ratio in feed, but ratios under 1:1 and over 7:1 lead to reduced growth and development [12, 13]. Magnesium is involved in the composition of bones, the construction and activation of important enzymes, the transmission of nerve impulses and the normal function of muscles. When there is a lack of Mg, Grass tetany is observed in ruminants [10]. Favorable quantitative relations between macro- and trace elements in feed increase their availability to animals.

Plants are the main natural source of mineral substances for humans and animals [8]. Legumes are richer in minerals than cereals [14, 15]. The low uptake efficiency by plants of some trace elements introduced through soil fertilization can be overcome by alternative methods such as foliar fertilization during vegetation [16, 17]. A number of authors reported that the application of organic fertilizers during vegetation affects the chemical composition by increasing the content of protein (N), fat, fibers and mineral substances in chickpea seeds [18-25]. According to Ansari *et al.* [26], liquid biofertilizers increased chickpea productivity but did not affect the N (protein) content of the grain. The lack of influence of various complex suspension fertilizers on the content of N (protein)

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was reported by Milev *et al.* [27] in soybean and Ilieva and Vasileva [28] in pea.

Rathod *et al.* [29] found that soil application of NPK along with foliar application of Zn and B increased the uptake of trace elements (Fe, Cu, Mn, Zn and B) in chickpea grain. Hidoto *et al.* [16] reported that foliar application of Zn increased the Zn content of chickpea grain by up to 22% compared to its soil application and pre-sowing seed treatment. Kobraee [30] observed an increased concentration of Zn and Mn in chickpea seeds and synergistic effects between these elements during their foliar application. An increased content of Zn and Fe in the chickpea grain after foliar application of mineral, chelated and nanoforms of Zn and Fe before flowering was also reported by Dhaliwal *et al.* [31] and Pal *et al.* [32].

The objective of this study was to determine the effect of liquid organic fertilizers Naturamin Plus and Amalgerol Essence on the mineral composition of chickpea grain. Some quantitative relations between macroelements in the grain were also established.

MATERIAL AND METHODS

Materials

The study was conducted in the period 2019-2021 in the region of Central South Bulgaria. The field experiment was conducted by the block method in 4 replications, size of the experimental area 10 m^2, under non-irrigated conditions, with a predecessor common wheat. The soil type was Haplic Vertisol, containing medium available humus, neutral to low alkaline reaction, low in available nitrogen and phosphorus and high in available potassium [33]. Meteorological conditions during the study period were characterized as relatively favorable [33]. Vegetation rainfall totals during the study years were above the multiyear average, but unevenly distributed. Average air temperatures for the chickpea vegetation period did not significantly differ from perennial averages.

The tested fertilizers had the following composition: Naturamin Plus (NP) – total 400 g/l amino acids, free amino acids – 200 g/l, N – 75 g/l, Fe – 12 g/l, Mn – 7.5 g/l, B – 1.3 g/l, Cu – 1.2 g/l, Mo – 0,5 g/l, Zinc (Zn) – 2.5 g/l; Amalgerol Essence (AE) – free amino acids, organic N (3%) and organic K (3%), plant herb extracts, seaweed extract, plant hormones, antioxidants, total organic carbon 22.7%. The fertilizers are certified according to European Council Regulation (EC) No 834/2007 for use in organic production.

The treatment was carried out in two phases of chickpea development: growth phase (4th leaf) and beginning of flowering with NP at doses of 1.5; 2.5 and 3.5 l/ha and AE at doses of 1.0; 2.0 and 3.0 l/ha.

Determination of mineral composition

The nitrogen content (N) was determined by the method of Kjeldahl (BDS – EN ISO 5983); phosphorus (P) – by colorimetry, measured at 470 nm on a SPEKOL 11 spectrophotometer; potassium (K); calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn) with Perkin Elmer AANALYST-800 atomic absorption spectrometer [38].

Statistical analysis

The results for the mineral composition were statistically processed with ANOVA LSD test for statistical significance of the differences, using MS Excel software – 2010.

RESULTS AND DISCUSSION

Fertilization with the liquid organic fertilizers NP and AE in 2019 (Table 1) increased the N content in the chickpea grain in both phases of application compared to the control variant.

The positive influence of fertilization is more pronounced at the beginning of the flowering phase, the highest values of N being recorded when treated with NP 3.5 l/ha and AE 1.0 l/ha. In 2021, again higher N content was observed at the beginning of the flowering phase - 38.74 g/kg when applying NP 3.5 l/ha and 39.38 g/kg when applying AE 3.0 l/ha.

On average for the study period, the higher N content in the grain due to NP fertilization at the 4th leaf phase was not statistically proven. In this phase, differences with the control were statistically proven only with AE at a dose of 1.0 l/ha ($P<0.05$). The positive influence of fertilization was more pronounced at the beginning of the flowering phase on average for the experimental period, and the differences from the control were statistically well proven when applying AE at doses of 1.0 and 3.0 l/ha ($P<0.001$) and NP 3.5 l/ha ($P<0.01$).

Of the macronutrients P, K, Ca and Mg included in this study (Table 2), potassium had the highest content in the chickpea grain. The established values for P content were higher than those for Ca and Mg. These results coincide with those obtained by Jukanti *et al.* [3] and Thavarajah and Thavarajah [15].

Table 1. Nitrogen content of chickpea grain, g/kg DM, n=39

Variant/Dose, l/ha	Years		Average	
	2019	2021	g/kg	%
Control (untreated)	35.58	38.06	36.82 ± 1.24	100.00
Growth phase (4th leaf)				
NP 1.5	36.49	37.69	37.09 ± 0.60[ab]	100.74
NP 2.5	36.93	37.52	37.22 ± 0.30[ab]	101.10
NP 3.5	36.49	36.69	36.59 ± 0.10[a]	99.37
AE 1.0	36.86	38.60	37.73 ± 0.87[*bc]	102.47
AE 2.0	37.09	37.46	37.28 ± 0.19[ab]	101.24
AE 3.0	36.99	37.14	37.06 ± 0.08[ab]	100.66
Average	36.81	37.52	37.16	
Beginning of flowering				
NP 1.5	36.84	37.32	37.08 ± 0.24[ab]	100.70
NP 2.5	36.28	38.62	37.45 ± 1.17[b]	101.71
NP 3.5	37.61	38.74	38.18 ± 0.56[**bc]	103.68
AE 1.0	37.60	38.93	38.27 ± 0.66[***c]	103.93
AE 2.0	37.23	36.82	37.02 ± 0.21[ab]	100.55
AE 3.0	37.32	39.38	38.35 ± 1.03[***c]	104.16
Average	37.15	38.30	37.72	

Different letters indicate statistically significant differences among variants at P<0.05; [*], [**], [***] – statistically significant differences between the variants and control at P<0.05, 0.01 and 0.001, respectively.

Table 2. Content of P, K, Ca and Mg of chickpea grain average for the period 2019-2021, g/kg DM, n=39

Variant/Dose, l/ha	P	K	Ca	Mg
Control (untreated)	3.70 ± 0.50	7.21 ± 0.37	2.11 ± 0.66	1.15 ± 0.11
Growth phase (4th leaf)				
NP 1.5	3.70 ± 0.70[ab]	7.25 ± 0.68[ab]	2.13 ± 0.73[a]	1.16 ± 0.17[bc]
NP 2.5	3.60 ± 0.50[a]	7.12 ± 0.61[a]	1.95 ± 0.68[a]	1.08 ± 0.07[b]
NP 3.5	3.70 ± 0.30[ab]	7.42 ± 0.29[ab]	2.00 ± 0.74[a]	1.14 ± 0.02[bc]
AE 1.0	4.05 ± 0.45[ab]	7.42 ± 0.21[ab]	2.00 ± 0.62[a]	1.05 ± 0.08[*ab]
AE 2.0	4.00 ± 0.70[ab]	7.18 ± 0.49[ab]	2.04 ± 0.68[a]	1.10 ± 0.13[bc]
AE 3.0	3.90 ± 0.40[ab]	7.29 ± 0.21[ab]	1.98 ± 0.69[a]	1.18 ± 0.05[bc]
Average	3.83	7.28	2.02	1.12
Beginning of flowering				
NP 1.5	3.80 ± 0.20[ab]	7.36 ± 0.10[ab]	2.02 ± 0.64[a]	0.96 ± 0.07[**a]
NP 2.5	4.05 ± 0.35[ab]	7.60 ± 0.27[b]	2.08 ± 0.68[a]	1.07 ± 0.08[b]
NP 3.5	4.15 ± 0.35[b]	7.67 ± 0.15[*b]	2.13 ± 0.70[a]	1.20 ± 0.11[c]
AE 1.0	3.70 ± 0.50[ab]	7.40 ± 0.10[ab]	2.01 ± 0.77[a]	1.07 ± 0.03[b]
AE 2.0	3.70 ± 0.20[ab]	7.21 ± 0.23[ab]	2.02 ± 0.68[a]	1.11 ± 0.02[bc]
AE 3.0	3.90 ± 0.40[ab]	7.07 ± 0.32[a]	1.95 ± 0.63[a]	1.11 ± 0.02[bc]
Average	3.88	7.38	2.03	1.09

Different letters indicate statistically significant differences between variants at P<0.05; [*], [**] – statistically significant differences between the variants and control at P<0.05 and 0.01, respectively.

Treatment with the organic fertilizer NP 3.5 l/ha at the beginning of the flowering stage increased provenly (P<0.05) the K content compared to the control by 6.5% and had the strongest positive effect on P, Ca and Mg content in the grain (by 12.2%, 0.9%, and 3.6%, respectively), but the differences from the control were not statistically proven. On average for the study period the lowest P, K and Ca content in the growth phase was reported for treatment with NP 2.5 l/ha, and at the phase beginning of flowering with AE, but the differences with the non-treated control were not proven.

A reduction in Mg content in the chickpea grain was reported in the growth phase after treatment with AE 1,0 l/ha (P<0.05) and at the beginning of the flowering phase for the variant fertilized with NP 1.5 l/ha (P<0.01).

For the trace elements studied in this research (Table 3), it is evident that fertilization with the organic fertilizers NP and AE has no significant effect on Fe, Cu and Mn content in chickpea grain.

Table 3. Content of Fe, Cu, Zn and Mn in chickpea grain average for the period 2019-2021, mg/kg DM, n=39

Variant/Dose, l/ha	Fe	Cu	Zn	Mn
Control (untreated)	58.97 ± 3.89	8.26 ± 1.59	29.90 ± 0.63	27.60 ± 8.22
Growth phase (4th leaf)				
NP 1.5	62.61 ± 2.81^{b}	8.51 ± 1.20	29.25 ± 0.80^{bc}	26.30 ± 8.46
NP 2.5	58.69 ± 2.40^{ab}	8.58 ± 2.20	29.71 ± 0.72^{c}	25.85 ± 8.44
NP 3.5	60.10 ± 6.35^{ab}	8.39 ± 1.04	30.00 ± 0.97^{c}	26.40 ± 7.47
AE 1.0	56.11 ± 5.47^{a}	8.23 ± 1.53	28.82 ± 0.08^{*b}	26.59 ± 7.02
AE 2.0	58.83 ± 6.97^{ab}	8.22 ± 1.17	29.46 ± 0.19^{c}	27.06 ± 6.73
AE 3.0	61.58 ± 4.05^{b}	8.22 ± 1.47	29.01 ± 0.86^{*bc}	27.40 ± 7.77
Average	59.65	8.36	29.37	26.60
Beginning of flowering				
NP 1.5	59.58 ± 5.64^{ab}	8.53 ± 2.45	27.80 ± 0.02^{***ab}	26.12 ± 7.87
NP 2.5	60.30 ± 2.21^{ab}	8.48 ± 2.22	27.90 ± 0.24^{***ab}	27.46 ± 7.30
NP 3.5	57.62 ± 5.25^{ab}	8.20 ± 1.71	29.47 ± 0.66^{c}	29.00 ± 7.70
AE 1.0	58.45 ± 2.82^{ab}	7.87 ± 1.20	28.54 ± 1.22^{**ab}	27.43 ± 6.71
AE 2.0	58.81 ± 3.90^{ab}	7.93 ± 1.69	28.60 ± 0.97^{**b}	27.16 ± 8.07
AE 3.0	59.91 ± 0.48^{ab}	8.26 ± 2.15	27.74 ± 0.88^{***a}	27.37 ± 7.42
Average	59.11	8.21	28.34	27.42

Different letters indicate statistically significant differences among variants at P<0.05; *, **, *** – statistically significant differences between the variants and control at P<0.05, 0.01 and 0.001, respectively.

Table 4. Influence of treatment with leaf fertilizers on the Ca:P ratio and Ca:Mg ratio average for the period 2019-2021

Variant/ Dose, l/ha	Growth phase (4th leaf)		Beginning of flowering	
	Ca:P	Ca:Mg	Ca:P	Ca:Mg
Control	1:1.76	1.83:1	1:1.76	1.83:1
NP 1.5	1:1.74	1.83:1	1:1.88	2.07:1
NP 2.5	1:1.84	1.81:1	1:1.95	1.95:1
NP 3.5	1:1.85	1.75:1	1:1.95	1.78:1
AE 1.0	1:2.02	1.91:1	1:1.84	1.88:1
AE 2.0	1:1.97	1.85:1	1:1.83	1.82:1
AE 3.0	1:1.97	1.69:1	1:2.00	1.75:1
Average	1:1.90	1.81:1	1:1.91	1.87:1

Higher content of the trace elements Fe, Cu and Zn was reported when fertilizing with NP at phase 4th leaf but no differences with the non-treated control were proven. Liquid organic fertilizer AE applied in phase 4th leaf resulted in reduction of Zn content by 3.0% – 3.6% compared to the control but the differences have a low degree of reliability (P<0.05). At the beginning of flowering phase treatment of chickpea with liquid organic fertilizers provenly reduced Zn content in the grain (P<0.001). The lowest Zn content was reported in treatment with AE 3.0 l/ha – 7.2% lower than the unfertilized control. The average values of Fe and Cu content (59.35 and 8.28 mg/kg, respectively), obtained in this study, are similar to the ones established by Jukanti *et al.* [3] and Thavarajah and Thavarajah [15]. The established Zn content is lower and that of Mn is higher than the ones reported by Jukanti *et al.* [3] and Thavarajah and Thavarajah [15].

Optimum Ca:P ratio in feed for ruminants is 2:1 (typical of green forages), although that ratio can vary a lot [10]. Ciepiela and Godlewska [34] found an increase of the ratios Ca:P and Ca:Mg in the vegetative application of organic biofertilizers in crops of grass species. According to Wolski *et al.* [35] foliar treatment with biostimulants reduced the Ca:P ratio in the biomass of three types of grass mixtures.

On average for the period of the present study, the Ca:P ratio in chickpea grain increased compared to the control variant (Table 4) as a result of the application of both foliar fertilizers. An increase is observed in the two treatment phases.

The average value is 1:1.90 (with variation from 1:1.74 to 1:2.02) and is close to that of Todorov *et al.* [36], who established a ratio of 1:2.1, unlike Iqbal *et al.* [37], who report a ratio Ca:P = 1:1.27 and

Thavarajah and Thavarajah [15], who report a ratio Ca:P = 1:2.55.

From data by Todorov *et al.* [36], the Ca:Mg ratio in chickpea grain is 2:1. The Ca:Mg ratio established in this study in chickpea grain is 1.84:1 on average, varying from 1.69:1 to 2.07:1. Higher, but not proven values of the Ca:Mg ratio (1.87:1 on average) were detected when chickpea was treated with the liquid fertilizers NP and AE at the beginning of the flowering phase.

CONCLUSIONS

Application of the liquid organic fertilizers Naturamin Plus and Amalgerol Essence to chickpeas has a proven positive effect on the N content in the grain. The highest content was found in treatment at the beginning of the flowering phase with Amalgerol Essence at a dose of 3.0 l/ha. The use of the liquid organic fertilizers Naturamin Plus and Amalgerol Essence does not significantly change the content of the other macroelements (P, K, Ca, Mg).

Liquid organic fertilizers Naturamin Plus and Amalgerol Essence do not affect the Fe, Cu and Mn content in chickpea grain. Treatment of chickpeas at the beginning of the flowering phase with Naturamin Plus and Amalgerol Essence results in a decrease of Zn content in the grain.

When fertilizing chickpeas with the liquid organic fertilizers Naturamin Plus and Amalgerol Essence, a tendency to increase the Ca:P ratio in the grain was observed in both phases of application. Fertilizers have a stronger effect on the Ca:Mg ratio when applied at the beginning of the flowering phase.

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