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Effect of Zinc Chelate and Sulfate on Mineral Content, Antioxidant Activity and Grain Yield of *Vigna unguiculata* L.

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The study evaluated the effect of applying different doses of zinc sulfate and zinc chelate on the mineral content, antioxidant activity, and yield of grains of cowpea bean (*Vigna unguiculata* L.). Doses of 7, 14, and 28 mM L^{-1} of zinc sulfate and chelate were assessed against a distilled water control. In the plants, the days to flowering as well as grain weight, number of pods, and number of grains per plant were recorded. Meanwhile, in the grains, antioxidant activity, phenols, phytic acid, and mineral content were determined. Results indicated that biofortification with 7 and 14 mM L^{-1} of sulfate and zinc chelate increases earliness in flowering, the number of grains per plant, and the grain yield in addition to improving the mineral content of the grains. The highest antioxidant activity was found with the zinc chelate treatments. Phenol content increased with the zinc chelate and sulfate doses, while the phytic acid content decreased with respect to the control. Biofortification of cowpea beans with zinc chelate and sulfate at 28 mM L^{-1} induced the highest accumulation of Zn in cowpea seeds. It is thus feasible to implement a biofortification program with zinc in cowpea beans to increase the zinc content, the mineral content, antioxidant activity, and phenol content in the grains, as well as to decrease the phytic acid content.

Key Words: phenols, phytic acid, *Vigna unguiculata* L., zinc chelate, zinc sulfate

INTRODUCTION

Zinc is an essential element in all organisms. In plants it is required for growth and development, as it participates directly in cell metabolism and forms a structural and functional part of many enzymes (Cakmak et al. 2017). It is also essential in photosynthesis and carbohydrate metabolism in plants because it stabilizes or activates proteins involved in these processes (Amezcua-Romero and Lara-Flores 2017). Therefore, its deficiency slows growth and reduces stress tolerance, chlorophyll synthesis, and yield (Sadeghzadeh 2013). It is estimated that around 2 billion people in the world have zinc deficiency (Gupta et al. 2014). Zinc deficiency has direct effects on growth, neurological development and the immune system (Cakmak and Kutman 2018) and is therefore considered a public health problem (Cakmak et al. 2017).

To increase the content of essential nutrients in plants, several strategies have been implemented. An example is biofortification, a process by which the content of essential nutrients is increased. The efficiency of this technique depends on the physiological capacity of the plant to accumulate the desired essential nutrient (Bouis et al. 2011). One of these nutrients is zinc, the absence of which limits crop yields (Mayer et al. 2008). In this regard, De Valença et al. (2017) mentioned that fertilization with zinc increases the yield and nutritional quality of crops, while Ghasal et al. (2018) and Márquez-Quiroz et al. (2018) reported that fertilization with zinc chelate and sulfate increases the zinc concentration in the edible parts of plants.

Cowpea (*Vigna unguiculata* L.) is one of the most important legumes in the world's tropical and subtropical regions (Carvalho et al. 2017) because it contains proteins, carbohydrates, vitamins, minerals, anthocyanins, essential amino acids, fibers, and phenolic compounds related to antioxidant activity (Chatterjee and Bandyopadhyay 2017; Kumari et al. 2015). Cowpea bean grains contain between 23.9 and 30.1 mg kg^{-1} of zinc (Manzeke et al. 2017), while 49–57 mg kg^{-1} of zinc has been reported in biofortified grains (Guillen-Molina et al. 2016). This study was conducted to evaluate the effect of applying different doses of zinc chelate and sulfate on the yield, antioxidant activity, and nutritional quality of cowpea bean grains.

MATERIALS AND METHODS

The study was carried out in the nursery and greenhouse area of the División Académica de Ciencias Agropecuarias at the Universidad Juárez Autónoma de Tabasco, located in the municipality of Centro, Tabasco, Mexico, at 17° 46′ 56″ NL and 92° 57′ 28″ WL, with an elevation of 21 m a.s.l. During the growing cycle, the average temperature was 30 °C, with a relative humidity of 80–90%. A 200 m^2 tropical Megavent-type protected structure, with a lateral anti-aphid mesh cover and ground cover mesh to prevent weed growth, was used. Planting of cowpea bean cv. Castilla was carried out in September 2018. Sowing was carried out in black polyethylene bags (25 cm wide x 30 cm high), which were filled with Tepetzil (porous stone of volcanic origin).

Fertilization was done with Hoagland and Arnon (1950) nutrient solution; the microelements were provided by TradeCorp AZ®. The pH was adjusted to 6.0 and the electrical conductivity to 2.0 dS m^{-1}. The nutrient solution was applied, providing 300 mL at 50% for the first 20 d after sowing (das) and then increasing to 1000 mL at 100% on day 21 until the end of the 90-d experiment. To wash the excess salts, 1500 mL of irrigation water was applied every 8 d. The water used was classified as C1S1 (Wilcox 1955). The treatments evaluated were 7, 14 and 28 mM L^{-1} doses of zinc sulfate ($ZnSO_4$) and zinc chelate (Zn-EDTA); distilled water was applied as control. The resulting seven doses obtained were applied at 21, 36, 51, 66, and 81 d after sowing. Initially, 25 mL was applied on the substrate of the plants, while 50 mL of the doses were applied in the subsequent applications.

Grain yield per plant was evaluated at 90 das (g), by adding up the weight of the grains harvested per plant in grams. Other variables assessed were days to flowering, the number of pods per plant, and the number of grains per plant, according to Guillen-Molina et al. (2016).

Methanol was used for the extraction of the phenolic compounds from the ground cowpea bean grains representing each dose. Total phenol content was determined with the Folin-Ciocalteau colorimetric method, as described by Singleton and Rossi (1965), which entails placing 0.5 mL of the methanolic phase in a test tube and then adding 1.5 mL of Na_2CO_3 at 2%, 0.5 mL of the Folin-Ciocalteau reagent at 50%, and 2.75 mL of deionized H_2O. After 1 h in the dark at room temperature, the absorbance at 725 nm was read on a Genesys 10S UV-Vis spectrophotometer. The calibration curve was made with caffeic acid solutions between 10 and 100 μg mL^{-1} with r^2 = 0.9997. The results are reported in milligrams of caffeic acid per gram of dry matter (mg CA g^{-1} DM).

Antioxidant activity was determined based on the methodology of Brand-Williams et al. (1995) through the capturing capacity of the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). One gram of cowpea bean flour was taken, mixed with 5 mL of methanol at 80%, and then centrifuged at 6000 rpm for 10 min. Next, 0.5 mL of the supernatant was taken and 2.5 mL of freshly prepared 0.1 mM DPPH solution was added. The mixture was incubated for 60 min in the dark and refrigerated. The absorbance reading of the mixture was taken at a wavelength of 517 nm in a Rayleigh model UV-1800 UV/VIS spectrophotometer. As a blank sample, 0.5 mL of methanol was used. The results are reported as percentage of DPPH inhibition with the formula:

$$\% \text{ inhibition} = (1 - (\text{absorbance of the mixture})/(\text{absorbance of the blank}))*100$$

Phytic acid content was determined with the enzymatic method reported by McKie and McCleary (2016), which is based on the principle of phytase hydrolyzation in phytic acid. The results were reported in grams of phytic acid per 100 g of sample dry weight (g PA 100 g^{-1} of sample DW).

Nitrogen (N), potassium (K), calcium (Ca), iron (Fe), and zinc (Zn) content was determined using the method described by Wolf (1982). Two grams of sample were mineralized by triacid digestion. K, Ca, Fe, and Zn were quantified by atomic absorption spectrophotometry in a Thermo ScientificTM spectrophotometer (iCE TM 3000 series AAS). Protein content was determined by multiplying the nitrogen content by a factor of 5.45 reported by Muranaka et al. (2016) for $V. unguiculata$.

Data were subjected to a simple ANOVA ($P \leq 0.05$). Means were compared by the orthogonal contrast test ($P \leq 0.05$). All analyses were performed with the statistical program SAS for Windows (SAS 2013).

RESULTS AND DISCUSSION

The 7 and 14 mM L^{-1} zinc chelate and sulfate doses induced early flowering and increased grain yield and number of pods and seeds per plant, while the 28 mM L^{-1} dose of both forms of zinc increased days to flowering and decreased grain yield and number of pods and seeds per plant (Table 1). In this regard, Quddus et al. (2011) indicated that total dry matter and yield increased in plants fertilized with zinc. Likewise, Moswatsi et al. (2013) and Subbaiah et al. (2016) reported that applying an adequate zinc dose improved biomass production and yield.

Table 1. Agronomic variables evaluated in cowpea beans (*V. unguiculata*) biofortified with different zinc chelate and sulfate doses.

Treatment	Dose (mM L^{-1})	Days to Flowering	Seed Yield (g per plant)	Pods per Plant	Seeds per Plant
1. Control	0	51.00 ± 1.00[a]	22.20 ± 3.57	14.33 ± 2.08	163.67 ± 13.58
2. Zn-EDTA	7	44.00 ± 1.00	38.47 ± 9.42	25.67 ± 4.16	164.33 ± 6.51
3. Zn-EDTA	14	44.00 ± 1.00	39.97 ± 2.71	26.67 ± 4.62	190.00 ± 8.19
4. ZN-EDTA	28	48.33 ± 3.06	27.53 ± 8.54	20.33 ± 5.51	162.33 ± 7.64
5. $ZnSO_4$	7	46.00 ± 2.65	30.20 ± 2.98	21.00 ± 2.00	204.00 ± 7.99
6. $ZnSO_4$	14	44.00 ± 1.00	31.57 ± 5.56	21.67 ± 4.04	183.67 ± 8.37
7. $ZnSO_4$	28	46.67 ± 1.53	28.07 ± 2.14	18.00 ± 1.00	190.00 ± 2.00
Contrast		**Treatment Comparisons (P-values of orthogonal contrast)**			
2+3+4 *vs* 5+6+7		0.0005**	<0.0001**	0.0008**	0.0359*
1 *vs* 2+3+4		0.2926	0.0003**	0.0687	0.0046**
1 *vs* 5+6+7		<0.0001**	<0.0001**	<0.0001**	0.3637
2 *vs* 5		0.0084**	0.0353*	0.6777	0.0013**
3 *vs* 6		0.0573	<0.0001**	0.0001**	1.0000
4 *vs* 7		<0.0001**	<0.0001**	0.0005**	0.0673

* ($p \leq 0.05$), ** ($p \leq 0.01$), [a]Mean ± standard deviation of five replications

Earliness in flowering as a result of application doses of both zinc chelate and zinc sulfate concurs with Singh et al. (2013) who indicated that fertilization with zinc causes precocity in plants. The highest grain yields, with 14 mM L^{-1} of zinc chelate (39.97 g per plant) and zinc sulfate (31.57 g per plant), were 80.04% and 42.21% higher, respectively, than the control yield. However, the yield decreased when zinc was increased to 28 mM L^{-1} in both zinc doses. Depending on the dose and the chemical forms of zinc, it can act as a nutrient, antioxidant, or even toxicant. In this regard, Quddus et al. (2011) indicated that yield is increased by increasing the zinc dose, until it reaches the dose at which yield decreases. The grain yields of zinc chelate and sulfate were within the range of grain yields reported by Márquez-Quiroz et al. (2015) for biofortified cowpea beans. The highest number of pods per plant, with the 14 mM L^{-1} zinc chelate dose (26.67), was 86.11% greater than the control. The number of pods per plant with the control and the zinc chelate and sulfate doses was higher than the 8.75 pods reported by Guillen-Molina et al. (2016) for biofortified cowpea beans. These differences may be due to the season of the year when the experiments were carried out. In the present study, the seeds were planted in the fall, while Guillen-Molina et al. (2016) planted in the spring, a season when temperatures were higher than those in the present study. High temperatures affect the flowering of cowpea beans, causing flower abortion and decreased yield (HanumanthaRao et al. 2016). For the number of grains per plant, there were values between 163.67 grains (control) and 204 grains (7 mM L^{-1} of zinc sulfate). This positive effect exerted by zinc on the number of grains per plant indicates a better efficiency in the growth processes and assimilate conversion, translocation and accumulation (Impa et al. 2013).

Antioxidant compounds are found in foods and have positive health effects due to their potential to protect people against reactive oxygen species (Wilson et al. 2017). The 7 mM L^{-1} zinc chelate dose (80.22%) resulted in the highest antioxidant activity, but when the dose was increased to 14 and 28 mM L^{-1} zinc chelate, the antioxidant activity decreased by 75.54% and 63.79%, respectively (Table 2). With the 7 and 14 mM L^{-1} zinc sulfate doses, the antioxidant activity increased by 56.24% and 66.07%, but with the 28 mM L^{-1} dose, it decreased by 61.27%. Previous studies have shown that biofortification with zinc improves the antioxidant capacity and nutritional quality of beans, but improvement depends on the abundance of metal ions (Zhu et al. 2013; Sida-Arreola et al. 2017). The contrast detected differences between the control and the zinc chelate and sulfate doses. When the zinc chelate and sulfate doses were compared, differences were observed between their respective 14 mM L^{-1} doses. The higher antioxidant activity as a result of applying 7 and 14 mM L^{-1} zinc chelate doses concurred with the findings of Sida-Arreola et al. (2017) who reported that zinc application in chelate form results in higher antioxidant activity, probably because zinc chelate is more efficiently absorbed by foliar and root applications (Rehman et al. 2012). Yuan et al. (2016) reported that the increase in zinc has effects on the antioxidant capacity and growth in peas. They also found that an increase from 10 to 50 μM zinc improved antioxidant capacity because zinc, chlorophyll, phenolic

Table 2. Antioxidant activity, total phenol content and phytic acid in cowpea beans (*V. unguiculata*) biofortified with different zinc chelate and sulfate doses.

Treatment	Dose (mM L^{-1})	Antioxidant Activity (DPPH Inhibition %)	Total Phenol Content (Caffeic Acid, mg g^{-1} DM)	Phytic Acid (g 100 g^{-1} sample)
1. Control	0	73.98 ± 3.10[a]	7.33 ± 0.24	1.74 ± 0.06
2. Zn-EDTA	7	80.22 ± 0.36	9.82 ± 2.22	1.15 ± 0.08
3. Zn-EDTA	14	75.54 ± 2.19	13.64 ± 3.52	1.34 ± 0.11
4. ZN-EDTA	28	63.79 ± 3.14	9.36 ± 0.09	0.91 ± 0.04
5. $ZnSO_4$	7	56.24 ± 14.23	12.80 ± 0.80	1.08 ± 0.06
6. $ZnSO_4$	14	66.07 ± 5.40	13.84 ± 2.92	0.82 ± 0.05
7. $ZnSO_4$	28	61.27 ± 9.89	15.20 ± 0.45	1.26 ± 0.17
Contrast		**Treatment Comparisons (P-values of orthogonal contrast)**		
2+3+4 *vs* 5+6+7		0.0089**	0.0808	0.1400
1 *vs* 2+3+4		0.0121**	0.0122*	0.0238*
1 *vs* 5+6+7		0.0140*	0.5467	0.0001**
2 *vs* 5		0.2271	0.0376*	0.0002**
3 *vs* 6		0.0325*	0.3096	0.0188*
4 *vs* 7		0.2066	0.0008**	<0.0001**

* ($p \leq 0.05$), ** ($p \leq 0.01$), [a]Mean ± standard deviation of five replications

content and amino acid content were increased, so that biofortification with zinc could increase the nutritional quality and antioxidant activity of legumes.

Phenolic compounds in foods have an important role in human health due to their antioxidant, anti-inflammatory, antiviral, and anticancer properties (Cevallos-Casals and Cisneros-Zevallos 2010). Total phenol content ranged between 9.36 and 13.64 mg of caffeic acid per gram of dry matter (mg CA g^{-1} DM) with the zinc chelate doses, and from 12.80 to 15.20 mg CA g^{-1} DM with the zinc sulfate doses, while the control had 7.33 mg CA g^{-1} DM. All zinc chelate and sulfate doses had higher phenolic content than the control. Therefore, increases in antioxidant activity of plants exposed to biofortification with zinc are mainly due to the increase in phenolic compounds, which are powerful scavengers of reactive oxygen species and are capable of inhibiting enzymes that produce free radicals (García-López et al. 2018). For the zinc chelate doses, the highest phenol content was obtained with the 14 mM L^{-1} dose; while for the zinc sulfate doses, the highest content was obtained with 28 mM L^{-1}. The highest phenol contents were obtained by applying zinc sulfate. The phenol contents obtained with the two ways of applying zinc were similar to the level reported by Sreerama et al. (2012) for cowpea beans.

Phytic acid is found in cereals, nuts, oilseeds and legumes in amounts ranging from 1% to 5%; it is an anti-nutrient that decreases the digestibility and bioavailability of cations (Chávez-Mendoza and Sánchez 2017). Although phytic acid is indigestible for humans and animals, it is important for plants and seeds due to its role in phosphorus and energy storage, with the undesirable property of limiting the availability of micronutrients (Oluwatosin 1999). Phytic acid content ranged from 0.91 to 1.34 g 100 g^{-1} of dry matter for the zinc chelate doses, and from 0.82 to 1.26 g 100 g^{-1} for the zinc sulfate doses. The phytic acid content of the different zinc chelate and sulfate doses was lower than the minimum value of 1.72 g 100 g^{-1} previously reported for cowpea beans (Oluwatosin 1999). In field trials, it has been shown that soil and/or foliar application of zinc fertilizers reduced grain phosphorus concentrations and thus grain phytate concentrations (Cakmak 2008). The zinc chelate and sulfate doses resulted in decreased phytic acid content compared with the control. This finding concurs with that of Erdal et al. (2002) who indicated that fertilization with zinc decreased phytic acid content, but such decreases could also be explained by the effect of dilution as a consequence of the higher grain yield with the different zinc chelate and sulfate doses (Sida-Arreola et al. 2017). Phytic acid at low concentrations is a natural antioxidant that has anticancer properties and prevents the formation of kidney stones as well as reduces serum cholesterol and triglycerides (Kumar et al. 2016). The contrasts detected differences between the control and the zinc chelate and sulfate doses, as well as differences among the 7, 14 and, 28 mM L^{-1} zinc chelate and sulfate doses. Zinc chelate and zinc sulfate decreased the phytic acid content, which improves the absorption capacity of zinc, iron, and calcium in people who consume cowpea beans biofortified with zinc.

Nutritional quality is an important issue to consider when discussing food. Plants provide many of the essential and nutritional compounds that contribute to better health. Increases in the contents of nutrients were observed with the two forms of zinc, with the zinc chelate doses producing the highest N, K, Ca and Zn content, and crude protein. The increase in mineral content with the 7 and 14 mM L^{-1} zinc chelate and sulfate doses indicates that applying zinc increases the mineral content of cowpea bean grains (Guillen-Molina et al. 2016). The protein content ranged between 22.55% and 23.86% (Fig. 1A), with the highest contents being produced by the 14 mM L^{-1} zinc sulfate (23.87%) and chelate (23.55%) dose. These crude protein contents are higher than those reported by Márquez-Quiroz et al. (2015) for biofortified cowpea beans, but they are low compared to the 21.00–30.70% content reported for a diversity of cultivated forms of *V. unguiculata* (Timko and Singh 2008). Furthermore, Yuan et al. (2016) found that the enrichment of zinc improved the nutritional quality as determined by the protein content and Zn content, indicating that biofortification with zinc improved nutritional quality, similar to the findings in our study. Nitrogen contents ranged between 4.14 and 4.38 g kg^{-1} (Fig. 1B), and values were within the 2.6–4.6 g kg^{-1} reported for cowpea beans (Carvalho et al. 2012). For potassium, there were increases (Fig. 1C) that were within the content levels reported for cowpea beans (Santos and Boiteux 2015). For calcium, values were between 0.08 and 0.9 g kg^{-1} with the zinc chelate and sulfate doses (Fig. 1D).

For iron, the 7 and 28 mM L^{-1} zinc chelate doses had increases of 1.00% and 2.71% with respect to the control, while for zinc sulfate, only the 28 mM L^{-1} dose yielded an increase in iron (Fig. 1E). The iron content obtained with the control and the zinc chelate and sulfate doses were within the 48–79 mg kg^{-1} range reported by Timko and Singh (2008). For zinc, the increases with respect to the control were 1.41%, 4.75%, and 11.44% with the zinc chelate doses, the increases were 3.17, 7.92, and 15.85% (Fig. 1F). In terms of the zinc contents with the 28 mM L^{-1} zinc chelate (63.30 mg kg^{-1}) and sulfate (65.80 mg kg^{-1}) doses, they were higher than the 61 mg kg^{-1} established as a critical zinc level (sufficient for human nutrition) in plants (Huett et al. 1997).

CONCLUSION

Biofortification of cowpea beans with 7 and 14 mM L^{-1} of zinc chelate and sulfate increased grain yield and number of pods and seeds per plant. The 7 mM L^{-1} zinc chelate dose produced the highest antioxidant activity. With regard to mineral content, increases were obtained with the two ways of applying zinc, but the highest N, K, Ca, Fe, Zn, and crude protein contents were found with application of zinc chelate. With respect to Zn accumulation in seeds, zinc biofortification induced the accumulation of this mineral in cowpea seeds, with both zinc forms applied. The highest zinc content in the cowpea bean grains was obtained in the 28 mM L^{-1} zinc chelate and sulfate dose.

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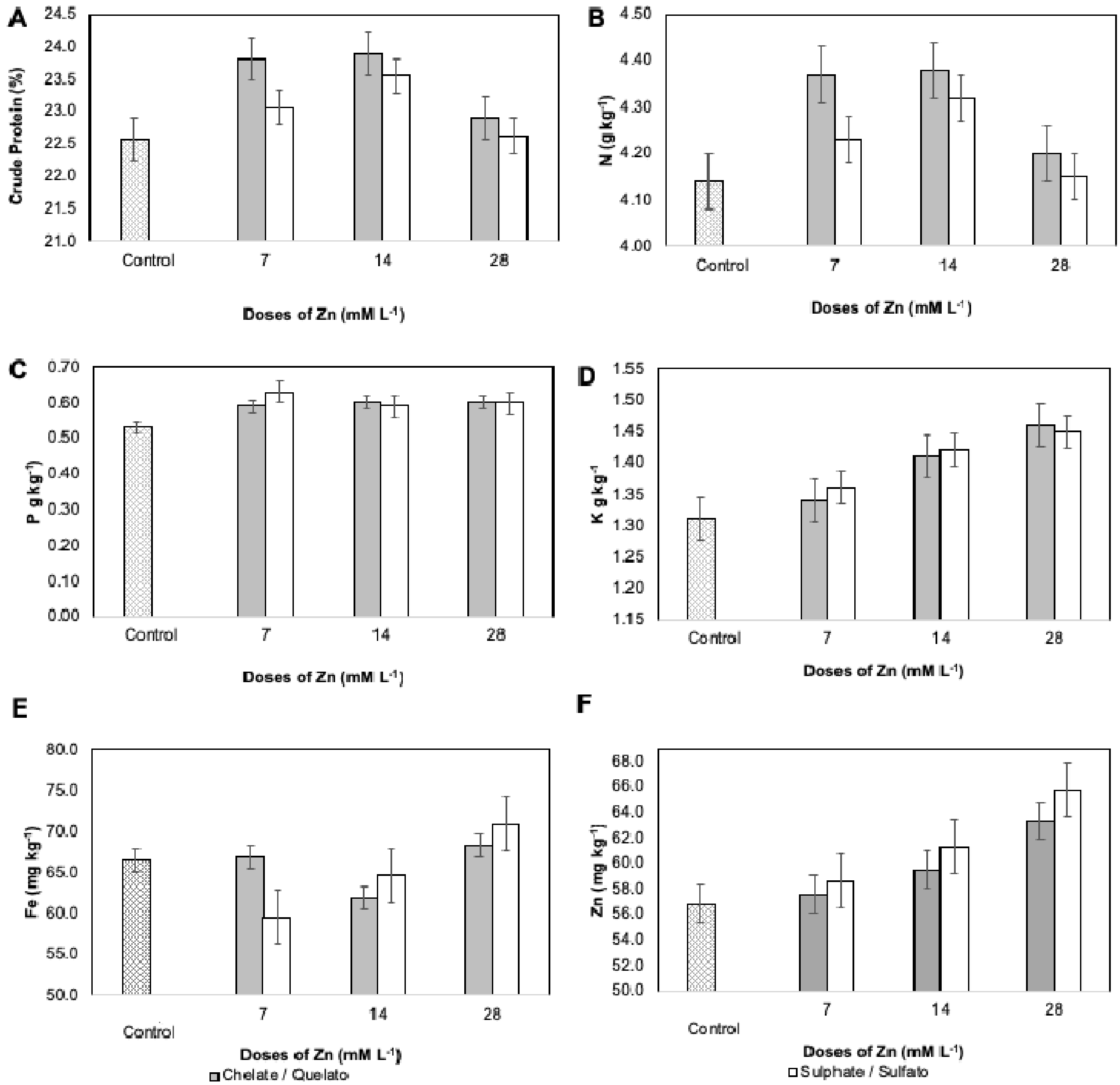

Fig. 1. Content of total crude protein (A) as well as (B) nitrogen, (C) phosphorus, (D) potassium, (E) iron, and (F) zinc minerals in the seed of cowpea beans (*V. unguiculata*) biofortified with different zinc chelate and sulfate doses.

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