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Biofortification of cowpea beans (*Vigna unguiculata* L. Walp) with zinc: effect on yield and mineral content

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Abstract

Zinc is an essential element in biological systems, which is required in small quantities. The objective was to know the effect of the foliar application of the doses of 0, 7, 14 and 28 mM L^{-1} of chelate (Zn-EDTA) and zinc sulfate ($ZnSO_4$) in the yield and mineral content of cowpea (*Vigna unguiculata* L.). In plants were counted the days to flowering, weight of grain, number of pods and number of grains per plant, in addition to the weight of 100 grains, while in the grains the content of total crude protein, nitrogen, phosphorus, potassium, calcium, magnesium, iron, zinc, boron, manganese, copper and molybdenum was determined. With the doses of 7, 14 and 28 mM L^{-1} of Zn-EDTA the yield of grain per plant with respect to the control was increased by 53.29, 41.58 and 38.42%, respectively, while with the same doses of $ZnSO_4$ the increase it was 27.80, 32.09 and 16.83%, respectively. The highest contents of nitrogen, crude protein, phosphorus, potassium, calcium, iron, manganese and copper were obtained with the doses of 7 and 14 mM L^{-1} of Zn-EDTA. While the highest levels of zinc, boron, molybdenum and magnesium were found with the doses of $ZnSO_4$, it was observed that the zinc content increases proportionally with the increase in the dose. The addition of different doses of Zn-EDTA and $ZnSO_4$ for the biofortification of cowpea beans has an effect on grain yield, crude protein content and mineral content.

Keywords: dosage, legumes, protein, chelates, sulfates.

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Introduction

The cowpea bean (*Vigna unguiculata* L. Walp) is an important source of food in the tropics and subtropics, provides adequate contents of protein, dietary fiber, carbohydrates, vitamins and phytochemicals (Devi *et al*., 2015). Dry beans, green pods, growing tips are consumed from cowpea beans and the whole plant is used as fodder (Márquez-Quiroz *et al*., 2015). For what is an important source of food for the rural population of the states of Campeche, Chiapas, Guerrero, Jalisco, Oaxaca, Tabasco, Veracruz, Yucatan and Tamaulipas (Lagunes-Espinoza *et al*., 2008; Apaez-Barrios *et al*., 2011).

Zinc is an essential micronutrient in biological systems, which is required in small quantities. It is involved in the formation and activation of enzymes that impact on the growth, development and production of plants (Poblaciones and Rengel, 2016). Its deficiency impacts on growth, pollen viability, flowering and grain production (Pandey *et al.*, 2006). In humans, its deficiency is associated with problems of growth and learning capacity in children, and increases the risk of infections, cancer and DNA damage (Ahmed *et al.*, 2014). It is present in around one third of the world population, which represents the fifth risk factor for diseases in developing countries (Shahzad *et al.*, 2014).

Biofortification is the agronomic process by which the content of elements and microelements in the edible parts of the cultivation plants is increased, which are applied in a foliar or edaphic form (Guillén-Molina *et al*., 2016). In this regard, Prasad *et al.* (2015) mention that the success of biofortification depends on the mobility of the element in the soil and the plant, zinc, is one of the most suitable microelements for biofortification, presenting its greatest absorption when applied as a foliar $ZnSO_4$. While Cakmak *et al*. (2010) report that the most effective method to increase the zinc content in the wheat grain is the edaphic and foliar application at the same time.

In cabbage (*Brassica oleracea* cv. Bronco) it is reported that the application of doses between 80 and 100 μM of Zn increase the concentration of zinc in the edible part of the plant (Barrameda-Medina *et al.*, 2017). While Sida-Arreola *et al*. (2015b) report that with 50 μM dose of zinc sulfate has the highest growth and yield of *Phaseolus vulgaris*.

Regarding cowpea beans, it is reported that the zinc content in the grain is low, especially when planted in soils with deficiencies of elements (Morales-Morales *et al.*, 2016). While Guillén-Molina *et al*. (2016) report that the zinc content in the cowpea bean grain is increased when it is biofortified with Zn chelate in an edaphic way. Therefore, the objective of this work was to know the effect of foliar application of different doses of zinc chelate (Zn-EDTA) and zinc sulfate ($ZnSO_4$) on the yield and mineral content of cowpea beans (*V. unguiculata* L.).

Materials and methods

Description of the study area

The work was carried out in the nurseries and greenhouses area of the Academic Division of Agricultural Sciences of the Universidad Juárez Autónoma of Tabasco, which is located in the municipality of Centro, Tabasco, Mexico at 17° 46' 56" north longitude and 92° 57' 28" west latitude, at an altitude of 21 m. We used a tropical Megavent type protected structure of 200 m^2, with lateral cover of anti-aphid mesh and Grown Cover mesh to prevent the growth of weeds.

Establishment of the crop

The sowing of cowpea beans (*V. unguiculata* L.) cv. De Castilla, was held on September 23, 2016. To have uniformity in germination, the seeds were submerged in water for one hour, then planted in black polyethylene bags 30 cm wide by 35 cm high, which They filled with porous white stone of volcanic origin (tepetzil). The planting density was 44 444 plants per hectare.

Fertilization and treatments

Fertilization was carried out with the nutrient solution of Hoagland and Arnon (1950) containing 14 mM of NO_3^-, 1 mM of $H_2PO_4^-$, 4 mM of SO_4^{2-}, 6 mM of K^+, 8 mM of Ca_2^+ and 4 mM of Mg^{2+}. To supply the microelements, we used the TradeCorp AZ® product that contains iron-EDTA 7.5% p/p, manganese-EDTA 3.4% p/p, zinc-EDTA 0.70% p/p, boron 0.68% p/p, copper-EDTA 0.28% p/p and molybdenum 0.26% p/p. The pH was adjusted to 6 and the electrical conductivity to 2 dS m^{-1}. The nutritive solution was applied manually, providing 250 ml of the nutritive solution at 50% the first 20 days after sowing (dds), and increasing to 1 000 ml of the 100% nutrient solution from day 21 to 90 dds

To wash the excess salts, an irrigation of 1 500 ml of water was applied every eight days. The irrigation water used was classified as C1S1 according to the U.S. Laboratory Salinity (Wilcox, 1955), which indicates that it has low salinity and sodium content, with C.E: 1.3 dS m^{-1}, pH: 7, cations (mM L^{-1}): Ca^{2+}= 4.6; Mg^{2+}= 13; K^+= 0.2; Na^+= 3 and anions (Mm^{-1}): HCO^{3-}=4.6; Cl^-= 4 and SO_4^{2-}= 0.

The treatments evaluated were the doses of 0, 7, 14 and 28 mM of zinc chelate (EDTA 14% p/p (Na_2Zn-EDTA) and zinc sulphate, hydrated ($ZnSO_4.7H_2O$), which resulted in eight treatments, which were evaluated in a completely randomized design with five replications. The treatments were sprinkled to the foliage of the plants with a 50 mL sprinkler, the first application was made at 21 dds, applying 25 mL of the dose, while in the applications performed at 36, 51, 66 and 81 dds were applied 50 mL per plant.

Management and phytosanitary control

The plants were guided and separated vertically from each other with raffia thread tutors. For control of atracnosis (*Colletotrichum lindemuthianum*) that occurred at 50 dds, Sulfacob 25® was applied at a dose of 3 kg ha^{-1}.

Agronomic variables evaluated

The grain yield per plant was evaluated at 90 dds, the grain weight of all the pods harvested per plant was added, days to flowering, the number of days elapsed from sowing to the appearance of the first flowers; number of pods per plant, the number of mature pods harvested at 90 dds, number of grains per plant, the sum of all the grains harvested per plant and weight of 100 grains, taking 100 grains at random and then weighing them in a granataria balance (Ohaus Scout® Pro) model H-2710 with an accuracy of ±0.1 g.

Mineral content analysis

The determination of the mineral content was made in the dry grains of the third and fourth pods of the harvested plants in each treatment, which were ground in a Krups Model GX4100 mill. The determination of the total nitrogen (N) content was made by the Micro-Kjeldahl method; to determine the content of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), boron (B), manganese (Mn), copper (Cu) and molybdenum (Mo), the 1 g of sample was mineralized by triacida digestion of each treatment. The P, was determined by the colorimetric method of ammonium metavanadate, and the K, Ca, Mg, Fe, Zn, Bo, Mn, Cu and Mo were quantified by atomic absorption spectrophotometry in a Thermo Scientific™ spectrophotometer (iCE ™ 3000 series AAS).

Statistical analysis

Statistical analyzes were performed under a completely randomized design. With the obtained means, the orthogonal contrasts test was carried out, all the analyzes were performed with the statistical program SAS 9.4 for Windows (SAS, 2013).

Results and discussion

Flowering in biofortified plants with different Zn-EDTA doses occurred between 46.0 and 51.0 dds, while plants biofortified with doses of $ZnSO_4$ flowered between 46.3 and 51.7 dds, while analysis of orthogonal contrasts detected statistical differences ($p \leq 0.01$) between the dose of 7 mM L^{-1} of $ZnSO_4$ and the doses with 14 and 28 mM L^{-1} (Table 1). Because flowering at all doses (treatments) occurred after 45 days, it is considered late (Manggoel and Uguru 2012). The different doses of Zn-EDTA and $ZnSO_4$, decreased the days to flowering, with a higher precocity of the plants treated with 7 mM L^{-1} of Zn-EDTA or $ZnSO_4$. For grain weight per plant values were found between 22.20 and 34.03 g per plant for Zn-EDTA doses, while $ZnSO_4$ doses had grain weights between 20.97 and 24.50 g, with statistical differences ($p \leq 0.01$) in the contrast of the control treatment (0 mM L^{-1}) and the doses of 7, 14 and 28 mM L^{-1} of $ZnSO_4$.

In general, these yields are greater than the 7 g reported by Apaez-Barrios *et al.* (2011) and the 13 g of grain per plant obtained by local producers of cowpea beans (SIAP, 2013); but they are equal to the average grain weights reported by Márquez-Quiroz *et al*. (2015) for biofortified cowpea beans. The highest performance of the treatments with the doses of 7, 14 and 28 mM L^{-1} of Zn-EDTA or $ZnSO_4$, coincides with that reported by Quddus *et al*. (2011) who indicate that the yield increases with increasing zinc dose.

Table 1. Days to flowering, yield and yield components in cowpea beans (*V. unguiculata* L.) biofortified with different doses of chelate and zinc sulphate.

Treatments	Days to flowering	Grain weight per plant (g)	Num. of pods per plant	Num. of grains per plant	Weight of 100 grains (g)
1) 0 mM L^{-1} Zn-EDTA	51 ±1	22.2 ±3.57	14.33 ±2.08	163.67 ±13.58	12.92 ±1.56
2) 7 mM L^{-1} Zn-EDTA	46 ±1	34.03 ±4.46	25.67 ±6.43	170.33 ±11.85	16.57±0.12
3) 14 mM L^{-1} Zn-EDTA	46.3 ±1.2	31.43 ±6.24	24.67 ±8.08	168.33 ±7.64	13.82 ±1.55
4) 28 mM L^{-1} Zn-EDTA	49.7 ±1	30.73 ±0.06	22.67 ±2.52	181.33 ±1.53	15.4 ±0.25
5) 0 mM L^{-1} $ZnSO_4$	51.7 ±1.5	20.97 ±2.11	15.33 ±3.51	164.67 ±4.73	12.5 ±1.6
6) 7 mM L^{-1} $ZnSO_4$	46.3 ±0.6	26.8 ±1.23	18.67 ±2.08	177.67 ±9.24	14.39 ±0.5
7) 14 mM L^{-1} $ZnSO_4$	46 ±1.7	27.7 ±3.84	19.67 ±2.31	172.67 ±8.08	12.9 ±2.63
8) 28 mM L^{-1} $ZnSO_4$	48.3 ±2.9	24.5 ±1.74	16.33 ±1.41	146.33 ±7.37	13.1 ±0.68
Orthogonal contrasts					
1 *vs* 2+3+4	0.804	0.051	0.044*	0.529	0.001**
2 *vs* 3+4	0.86	0.317	0.139	0.111	0.076
3 *vs* 4	0.761	0.246	0.073	0.387	0.051
5 *vs* 6+7+8	0.159	0.008**	0.005**	<.001**	0.003**
6 *vs* 7+8	0.009**	0.36	0.655	0.003**	0.926
7 *vs* 8	0.607	0.618	0.968	0.355	0.362

*= ($p \leq 0.05$), **= ($p \leq 0.01$).

The number of pods per plant ranged between 14.33 and 25.67 for the different doses of Zn-EDTA, and from 15.33 to 19.67 for the doses of $ZnSO_4$. These values are higher than the 8.75 pods obtained by Guillén-Molina *et al*. (2016) for cowpea beans biofortified with zinc. The differences may be due to the time of year in which the experiments were planted, since the present work was planted in the autumn winter cycle, while the work of Guillén-Molina *et al*. (2016) was sown in the spring-summer cycle, with average temperatures higher than those presented in the present work. The effect of temperature on the flowering of cowpea beans is known to be sensitive to high temperatures in the reproductive stage, which causes flower abortion and decreased yield (Hanumantha *et al*., 2016).

The number of grains per plant ranged between 163.67 and 181.33 grains for the doses of Zn-EDTA, and from 164.67 to 177.33 grains for the doses of $ZnSO_4$. For the weight of 100 grains values between 12.92 and 16.67 g were found for the different doses of Zn-EDTA, while the doses of $ZnSO_4$ had weights of 100 grains between 12.50 and 14.39 g. Observing statistical differences ($p \leq 0.01$) between the control doses and the different doses of Zn-EDTA and $ZnSO_4$ (table 1). On average, the doses of 7, 14 and 28 mM L^{-1} of Zn-EDTA had a 28.25, 6.97 and 19.20% greater weight of 100 grains than the control (0 mM L^{-1}), while the doses of 7, 14 and 28 mM L^{-1} of $ZnSO_4$ had weight increases of 100 grains of 15.12, 3.20 and 4.80% with respect to the control.

In the treatments with Zn-EDTA and $ZnSO_4$, the 7 mM L^{-1} dose with the highest average weight of 100 grains stands out. In general, the weights of 100 grains of all the treatments are higher than the 12.0 and 12.4 g reported by Lagunes-Espinoza *et al*. (2008) and Apaez-Barrios *et al*. (2011), but according to Márquez-Quiroz *et al*. (2015) only the doses with 7 and 28 mM L^{-1} of Zn-EDTA had the higher weights of 100 grains of biofortified cowpea beans.

The doses of 7, 14 and 28 mM L^{-1} of Zn-EDTA and $ZnSO_4$ decreased the days to flowering, and increased the weight of grain, number of pods per plant, number of grains per plant and the weight of 100 grains, which indicates that the different doses Zn-EDTA and $ZnSO_4$ increase the production of biomass and grain in the plants of cowpea beans, which coincides with Ratto and Miguez (2005) and Quddus *et al*. (2011) who indicate that total dry matter and yield increase in plants biofortified with zinc, while Sida-Arreola *et al*. (2015a) and Ibrahim and Ramadan (2015) indicate that the application of the adequate dose of Zn increases biomass and yield.

The total nitrogen (N) content ranged between 4.14 and 4.38% for the doses of Zn-EDTA, while for the doses of $ZnSO_4$ it was 4.15 to 4.32% (Figure 1B). These values are higher than those reported by Márquez-Quiroz *et al*. (2015) and Guillén-Molina *et al*. (2016). According to the factor of 5.45 to convert the nitrogen content to crude protein of *V. unguiculata* L. (Muranaka *et al*., 2016), the protein content ranged between 22.56 and 23.87% for the different doses of Zn-EDTA and from 22.62 to 23.54% for the doses of $ZnSO_4$ (Figure 1A); presenting the highest total crude protein contents at 14 mM L^{-1} doses of $ZnSO_4$ (23.89%) and Zn-EDTA (23.54%). These crude protein contents are higher than the 20.3% reported by Guillén-Molina *et al*. (2016) and 22% of protein reported by Márquez-Quiroz *et al*. (2015) for biofortified cowpea beans.

But they are low with respect to the maximum value of the total crude protein range of 21 to 30.70% reported for *V. unguiculata* (Timko and Singh, 2008). The highest contents of total crude protein were obtained with the doses of 7 and 14 mM L^{-1} of Zn-EDTA (23.82 and 23.87%) and $ZnSO_4$ (23.05 and 23.54%), decreasing the content of crude protein in 4.28% in the dose of 28 mM L^{-1} of Zn-EDTA and 4.06% in the dose of 28 mM L^{-1} of $ZnSO_4$. What can be due to higher doses of Zn cause oxidative stress to the plant, which increases the production of active oxygen species, which damages the cellular components and the production of proteins (Muhammad *et al*., 2016).

It can also be due to the problem of toxicity that occurred with the application of 28 mM L^{-1} doses of Zn-EDTA and $ZnSO_4$ at 21 dds, manifesting with problems of leaf necrosis and reduced plant growth. With the doses of 14 mM L^{-1} of Zn-EDTA and of $ZnSO_4$, the highest content of nitrogen and total crude protein (Figure 1A) and the lowest grain yields per plant were observed (Table 1), which may be due to the negative correlation between nitrogen content and grain yield (Olunike, 2014).

When comparing the phosphorus content (P) of the control with the doses of 7, 14 and 28 mM L^{-1} of Zn-EDTA, increments of 18.46, 18.46 and 1.85% are observed, respectively; while the doses of 7, 14 and 28 mM L^{-1} of $ZnSO_4$ had increases of 20.75, 20.75 and 18.86%, respectively (Figure 1C). There was a slight decrease in phosphorus with the doses of 28 mM L^{-1} of Zn-EDTA and $ZnSO_4$, which indicates that with the zinc doses used, the maximum limit was not reached to observe the pronounced decrease in phosphorus reported by Cakmak *et al*. (2010).

For the content of potassium (K) it was found that, with the doses of 7, 14 and 28 mM L^{-1} of Zn-EDTA was increased by 6.87, 7.63 and 7.63%, respectively, the potassium content; while the doses of 7, 14 and 28 mM L^{-1} of $ZnSO_4$ the potassium content increased by 0, 3.82 and 7.63%, respectively (Figure 1D).

The contents of potassium found in all doses are within the contents reported for cowpea beans (Frota *et al*., 2008; Márquez-Quiroz *et al*., 2015). With regard to the content of calcium (Ca), values between 0.08 and 0.9% were found in the different doses of Zn-EDTA and $ZnSO_4$ (Figure 1E), with increases in the calcium content with the doses of 7 and 14 mM L^{-1} of Zn-EDTA of 12.5%, while with $ZnSO_4$ the decrease in calcium content was observed with the dose of 28 mM L^{-1}. For the content of magnesium (Mg) values between 0.22 and 0.25% were found with the doses of Zn-EDTA and 0.24 and 0.25% with the doses of $ZnSO_4$ (Figure 1F), observing that with the dose of 14 mM L^{-1} of Zn-EDTA and $ZnSO_4$ have the highest Mg contents, while with the dose of 28 mM L^{-1} of $ZnSO_4$ there is a decrease of 8.33% with respect to the control.

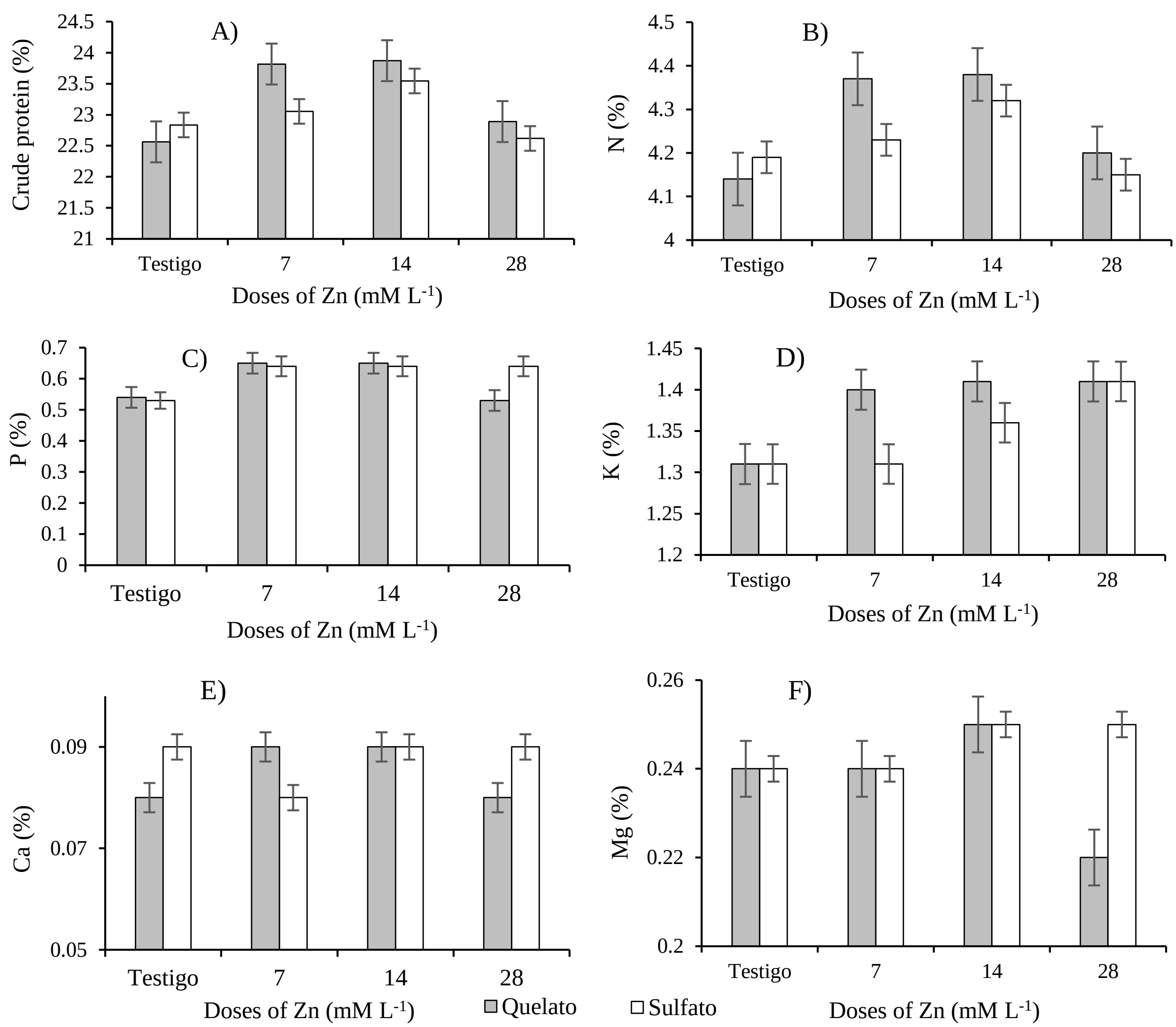

Figure 1. Content of total crude protein (A), nitrogen (B), phosphorus (C), potassium (D), calcium (E) and magnesium (F) in the bean seed of cowpea (*V. unguiculata* L.) biofortified with different doses of chelate and zinc sulfate.

The increase in mineral contents with the doses of 7 and 14 mM L^{-1} of Zn-EDTA and $ZnSO_4$, indicate the influence of biofortification with zinc on the mineral content of cowpea beans (Guillén-Molina *et al.*, 2016). For the content of crude protein and macroelements, it was found that the highest contents were obtained with doses of 7 and 14 mM L^{-1} of Zn-EDTA and of $ZnSO_4$, but with a tendency to present the highest values at doses of 7 and 14 mM L^{-1} of Zn-EDTA, which may be due to the greater assimilation of the chelated fertilizers and that some elements are characterized by being inside the plant in a chelated form (Sánchez, 1984).

For the content of iron (Fe) it was observed that with the doses of 7 and 14 mM L^{-1} of Zn-EDTA there were increases of 11.14 and 9.19% of the iron, respectively; while with doses of $ZnSO_4$, only with 7 mM L^{-1} there was an increase of 0.71% of iron. Meanwhile, with the doses of 28 Mm L^{-1} of Zn-EDTA and $ZnSO_4$, the iron content decreases 4.37 and 14.61% (Figure 2A). Similar effects have been observed in *V. unguiculata*, with reports indicating that doses greater than 15 mM L^{-1} of $ZnSO_4$ decreases iron content by 31.10% (Morales-Morales *et al.*, 2016). In general, iron content is within the range of 48 to 79 ppm reported for *V. unguiculata* (Timko and Singh, 2008). For the zinc content there were increases of 5.81, 8.98 and 2.11% with the doses of 7, 14 and 28 mM L^{-1} of Zn-EDTA, while the doses of $ZnSO_4$ had increases of 11.28, 19.99 and 23.61%, respectively (Figure 2B).

The higher zinc contents found in the present work with respect to what was reported by Timko and Singh (2008) and Guillen-Molina *et al.* (2016), it can be because the foliar application of zinc is more effective than the edaphic application, to increase its content in the grain (Yue-Qiang *et al*., 2012). The higher zinc content in the biofortified grains with the $ZnSO_4$ doses, may be due to the biofortification with $ZnSO_4$ in foliar form is more effective to increase the zinc content in the grain, than that made with Zn-EDTA (Prasad *et al*., 2015). The increase in Zn and the decrease in Fe, with the increase in the dose of Zn-EDTA and $ZnSO_4$, is due to the antagonistic effect between Zn and Fe (Fasaei and Ronaghi, 2015).

Regarding Shekari *et al*. (2015) report that the increase in the dose of Zn, decreased the Fe content in the wheat grain. With the doses of 7, 14 and 28 mM L^{-1} of $ZnSO_4$ and with 14 mM L^{-1} of Zn-EDTA there were 64.10, 68.00, 71.20 and 61.90 ppm, values that are higher than the 61 ppm established as critical level of zinc in plants, to have a zinc sufficiency status in human nutrition (Huett *et al*., 1997).

The boron content was 16.90 to 21.10 ppm for the doses of Zn-EDTA, while for the $ZnSO_4$ doses the boron content was 20.60 to 23 ppm (Figure 2C). For the different doses of Zn-EDTA it is observed that the control (0 mM L^{-1}) had the highest boron content, while the doses of 7, 14 and 28 mM L^{-1} decrease 15.17, 3.32 and 19.91% on the boron content. Whereas the doses of 7, 14 and 28 mM L^{-1} of $ZnSO_4$ increased the boron content by 1.94, 3.99 and 11.65%. For the manganese content values between 20.30 and 26.70 ppm were found for the doses of Zn-EDTA, while in the doses of $ZnSO_4$ the content was 18.70 to 19.40 (Figure 2D).

The copper content ranged between 10.90 and 17.60 ppm for the different doses of Zn-EDTA, and from 9.84 to 15.20 ppm for the doses of $ZnSO_4$ (Figure 2E), with increases of 61.47, 53.21 and 4.59% in the doses of 7, 14 and 28 mM L^{-1} of Zn-EDTA, while, in the doses of 7, 14 and 28 mM L^{-1} of $ZnSO_4$ the increase was of 54.47, 49.39 and 16.87%. In general, it is observed that the copper content increases with the doses of 7 and 14 mM L^{-1} of Zn-EDTA and of $ZnSO_4$, but decreases with the doses of 28 mM L^{-1}. For the molybdenum content values between 10 and 15.10 ppm were found in the doses of Zn-EDTA, while the $ZnSO_4$ doses had contents of 13.40 to 17.70 ppm (Figure 2F).

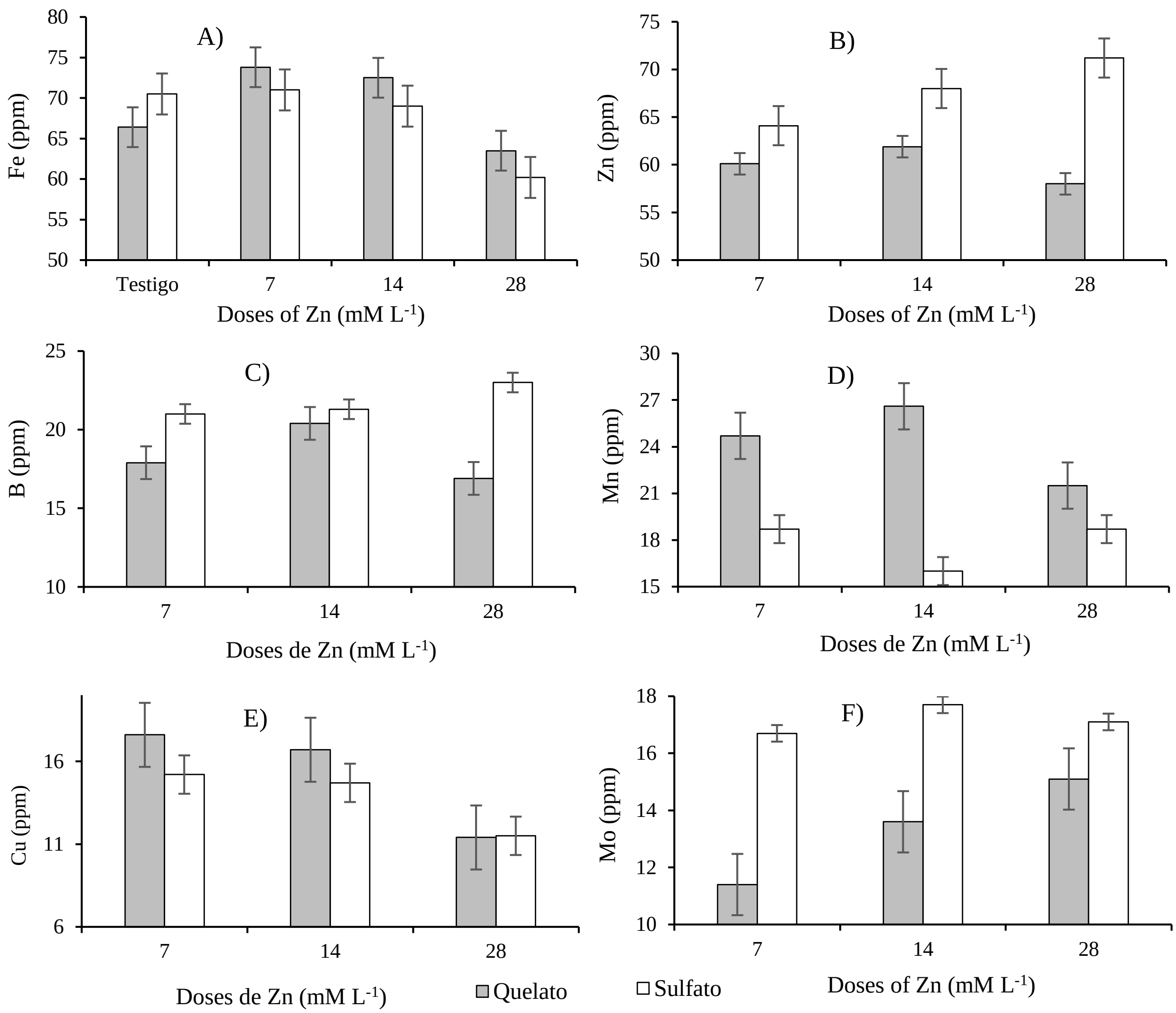

Figure 2. Content of iron (A), zinc (B), boron (C), manganese (D), copper (E) and molybdenum (F) in seeds of cowpea beans (*V. unguiculata* L.) biofortified with different doses of chelate and zinc sulfate.

Conclusions

The biofortification of cowpea beans with different doses of Zn-EDTA and $ZnSO_4$ have effects on grain yield, total crude protein content and mineral content. The doses of 7, 14 and 28 mM L^{-1} of Zn-EDTA had the greatest increases in grain yield per plant with respect to the control. The doses of Zn-EDTA and $ZnSO_4$, decreased the days to flowering, and increased the number of pods, number of grains and the weight of 100 grains with respect to the control. The highest contents of nitrogen, crude protein, phosphorus, potassium, calcium, iron, manganese and copper were obtained with the doses of 7 and 14 mM L^{-1} of Zn-EDTA.

The zinc content increased proportionally with the doses of 7, 14 and 28 mM L^{-1} of $ZnSO_4$ in 11.28, 19.99 and 23.61% presenting the doses of 7, 14 and 28 mM L^{-1} of $ZnSO_4$ and the dose of 14 mM L^{-1} of Zn-EDTA values higher than 61 ppm, which is considered as critical value to have a zinc sufficiency status in the plants cultivated for human consumption.

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