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Effect of nutrient omission in the development of sunflower BRS-122 in greenhouse conditions

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Efecto de la omisión de nutrientes en el desarrollo de girasol cultivar BRS-122 en condiciones de invernadero

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Allan Nunes Alves[1], Felipe Guedes de Souza[1], Lúcia Helena Garófalo Chaves[1]*, Jorge Alves de Sousa[1] and Ana Carolina Feitosa de Vasconcelos[1]

Keywords:
Helianthus annuus
Missing mineral nutrients
Nutrient solution
Visual diagnosis

ABSTRACT

Sunflower (*Helianthus annuus* L.) is responsible for 13% of all vegetable oil produced in the world. These plants' development depends on the mineral elements that have essential and specific functions in their metabolism. In this sense, visual diagnosis consists of comparing the appearance of a plant that has received all the necessary nutrients with one that has suffered the omission of one or more nutrients. Therefore, this study aimed to evaluate the absence effects of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S) and iron (Fe) elements on the growth of sunflower, BRS-122 cultivar, in order to identify and describe the visual symptoms caused by the absence of such nutrients. The experiment was carried out in a greenhouse and consisted in a completely randomized design with three replications and eight treatments using a diagnostic subtraction technique. The symptoms of the deficiencies were observed and evaluated through biometric parameters (plant height, stem diameter, number of leaves, and leaf area) as well as by visual aspects. The absence of N, P, K, Ca and Fe in the nutrient solution severely affected the sunflower plants, preventing their vegetative growth and consequently their development. The negative interference of the Mg omission in sunflower growth was slower than the observed for nitrogen, phosphorus, potassium, and calcium giving more significant results from 40 days after sowing (DAS). The absence of nutrients gave clear evidence of the distinct effects that the omission of each element can cause on the visual aspects of sunflower plants.

Palabras clave:
Helianthus annuus
Nutrientes minerales faltantes
Solución nutritiva
Diagnóstico visual

RESUMEN

El girasol (*Helianthus annuus* L.) es responsable del 13% de todo el aceite vegetal producido en el mundo. El desarrollo de estas plantas depende de los elementos minerales que tienen funciones esenciales y específicas en su metabolismo. En este sentido, el diagnóstico visual consiste en comparar la apariencia de una planta que recibió todos los nutrientes necesarios con una que sufrió la omisión de uno o más nutrientes. Por lo tanto, este estudio tuvo como objetivo evaluar los efectos de la ausencia de nitrógeno (N), fósforo (P), potasio (K), calcio (Ca), magnesio (Mg), azufre (S) y hierro (Fe) en el crecimiento de girasol, cultivar BRS-122, a fin de identificar y describir los síntomas visuales de la ausencia de tales nutrientes. El experimento fue conducido en invernadero y consistió en un diseño completamente al azar, con tres repeticiones y ocho tratamientos, utilizando una técnica de diagnóstico de sustracción. Los síntomas de las deficiencias fueron observados y evaluados a través de parámetros biométricos (altura de la planta, diámetro del tallo, número de hojas y área foliar), así como por aspectos visuales. La ausencia de N, P, K, Ca y Fe en la solución nutritiva afectaron severamente a las plantas de girasol, impidiendo su crecimiento vegetativo y consecuentemente su desarrollo. La interferencia negativa de la omisión del Mg en el crecimiento del girasol fue más lenta que la observada en N, P, K y Ca, con resultados más significativos a partir de los 40 días después de la siembra (DAS). La ausencia de los nutrientes dio claras evidencias de los distintos efectos que la omisión de cada elemento puede causar en los aspectos visuales de las plantas de girasol.

[1] Universidad Federal de Campina Grande. R. Aprígio Veloso, 882 - Universitário Campina Grande. PB 58429-900, Paraíba, Brasil.
* Corresponding author: <lhgarofalo@hotmail.com>

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Sunflower (*Helianthus annuus* L.) is an annual plant, originally from the American continent; however, it is grown all over the world. The expansion of its cultivation in all Brazil regions is due to its good adaptation to diverse edaphoclimatic conditions, being characterized by the tolerance to low temperatures in the initial phase of development and by the relative resistance to water deficits. According to Zobiole (2010), the sunflower yield is influenced by latitudes and altitudes as well as by the photoperiod.

The sunflower is responsible for 13% of all vegetable oil produced in the world, which it is currently the fourth most consumed oil in the world after soybean, palm, and canola. Seeds are rich in oil, some sunflower varieties produced by hybridization have amounts higher than 50%, they rarely contain less than 30% (Lira *et al*., 2011). This oil has excellent industrial and nutritional quality, being its primary use as edible oil (Castro *et al*., 1997). Besides, it is an extremely versatile plant, it can be used for animal feed, in human food, and as an ornamental plant (Rodrigues *et al*., 2010).

Mineral elements, such as macronutrients, have essential and specific functions in plant metabolism. Thus, when one of these elements is not present in adequate amounts, or under conditions that make it unavailable, its deficiency in cells promotes changes in plant metabolism manifested by characteristic deficiency symptoms (Taiz and Zaiger, 2009). Therefore, it is recommended to study the effects caused by the lack of mineral elements on sunflower culture, since it has economic relevancy; besides, adequate mineral nutrition of plants is crucial for ideal growth of plants.

For example, Nitrogen (N) is part of the amino acids, proteins, nucleic acids, enzymes, and pigments structure, and it participates in processes of photosynthesis, respiration, multiplication and cellular differentiation (Malavolta *et al*., 1997; Marschner, 1995). Phosphorus (P) participates in the structural formation of plants, in the energy supply to produce photoassimilates and in the quality of final products (Brandão, 2009). Potassium (K) acts directly and indirectly in photosynthesis and respiration, as well as in the food plant transportation. Among nutrients, K is described as having a significant influence in combating plant diseases, as it increases resistance to some pathogens development, it also increases cell wall thickness, provides greater tissue stiffness and promotes rapid recovery after injury (Basseto *et al*., 2007). Calcium (Ca) influences elongation and differentiation of cells (Bergmann, 1992). Ca deficiency can cause meristem death (Marschner, 1995). From the existing nutrients, magnesium (Mg) is essential in photosynthesis because it participates in metabolic processes such as ATP formation in chloroplasts. Magnesium also acts in protein synthesis, chlorophyll formation, phloem loading, photoassimilates separation and use (Marschner, 1995). Sulfur (S) is a secondary anionic macronutrient necessary for the of plants development. S functions are hormonal control for cell growth and differentiation, it supports the plant defense against pests and diseases, and it is an important component for proteins. Iron (Fe) is a micronutrient that acts as an enzyme activator or component, influences the fixation of nitrogen, catalyzes the biosynthesis of chlorophyll, and acts on stems and roots development (Malavolta *et al*., 1997; Marschner, 1995).

Visual diagnosis is used to understand the element absence on plant development. It consists of evaluating and comparing the appearance of a plant that received a solution, with all the necessary nutrients, to another plant that received a solution missing one or more nutrients. In most cases, the leaf appearance is generally analyzed, but it can be analyzed elsewhere in the plant depending on the element absented (Carvalho *et al*., 2001; Malavolta *et al*., 1997). However, before the visible manifestation of the nutrient deficiency, growth and/or production can already be affected by this deficiency, this is what is called hidden hunger, which can only be detected through chemical analysis of the plant material or foliar diagnosis (Malavolta, 2006).

On these bases, the objective of this research was to evaluate the effects of macronutrients and micronutrient (iron) absence on the growth of sunflower, BRS-122 cultivar, and to identify and describe the visual symptoms caused by such nutrients absence.

MATERIAL AND METHODS

The experiment was carried out under greenhouse conditions at the Department of Agricultural Engineering of Universidade Federal de Campina Grande, from June

to August of 2016, with sunflower plants of the BRS-122 cultivar.

The statistical experimental design was completely randomized, consisting of eight treatments and three replicates, totaling 24 experimental units. Each experimental unit presented a sunflower plant, according to the following treatments: T1- control treatment with the complete solution (CS) according to Hoagland and Arnon (1950), T2- nutritive solution with omission of nitrogen (N), T3- nutritive solution with omission of phosphorus (P), T4-nutritive solution with omission of potassium (K), T5- nutritive solution with omission of calcium (Ca), T6- nutritive solution with omission of magnesium (Mg), T7-nutritive solution with omission of sulfur (S), and T8- nutritive solution with omission of iron (Fe). The only micronutrient evaluated was Fe because, in a previous pilot test designed to define the treatments of this research, it was observed that the omission of the other micronutrients (B, Cu, Mn, Mo, and Zn) did not show symptoms of deficiency in sunflower plants.

The stock nutrient solutions (control) were prepared with guaranteed reagents and deionized water. During the whole experiment, pH and electrical conductivity (EC) measurements were taken to control them, always maintaining pH values in the range of 6.0 to 7.0 and EC around 2.5 dS m^{-1}.

The sunflower plants BRS-122 cultivar used in the experiment were obtained via seeds germinated in phenolic sponges conditioned in a plastic container (disposable cups) with a capacity of 50 mL containing deionized water until the surface of the sponge. Six days after germination, when the formation of four leaves in the seedlings was observed, they were transferred to one-liter pots with the complete nutrient solution established by Hoagland and Arnon (1950), but only with 10% of the ionic strength for the seedlings' adaptation and under constant aeration. In the sequence, the ionic strength of the solution increased to 40% in the second week, increasing gradually up to 100%. After this adaptation period (three weeks), plants were transplanted to one-liter pots, and the treatments were applied under the missing element technique. The pots were capped with expanded polystyrene with a hole in the center of them for the fixation of the crops and another hole in the end for the air entrance.

The evaluations of biometric characteristics such as plant height, stem diameter, and the number of leaves were done at 20, 30, 40 and 50 days after sowing (DAS). The leaf area was evaluated only in the last three samplings. At 50 DAS, plants were harvested and separated in roots and shoot (leaves, branches, and stems) packed in paper bags properly labeled and taken for drying in a forced circulation oven at 65 °C until reaching constant weight, obtaining the dry phytomass weight of roots and shoot.

Visual deficiency symptoms were initially recorded at 20 DAS by photographs and daily described throughout the experimental period (50 DAS) in order to observe the beginning of each deficiency symptom. The data were submitted to analysis of variance and comparison of means, using the Tukey test at 1% of probability level, applying the SISVAR software (Ferreira, 2011).

RESULTS AND DISCUSSION

Biometric Characteristics Assessments

The nutritional omissions that affected the most the plant height variable, restricting it, were: Ca, Fe (75.77, 65.43%, respectively) at 30 DAS; K, N, P, Mg, and S (77.05, 71.01, 61.84, 27.54%, and 18.84%, respectively) at 50 DAS, when compared to the complete treatment (control). Due to Ca omission in the nutrient solution, the plants were developed only up to the 30 days of omission, presenting the shortest height comparing to the control treatment, dying after this period (Figure 1). These effects were similar to the iron absence in the nutrient solution of plants.

The plants submitted to treatments with the omission of N, P, and K, maintained a continuous growth throughout the experiment, presented plant height inferior to the control treatment, with heights 3.45, 2.62, and 4.36 times lower than the observed for the control treatment, respectively. The solutions with the omission of Mg and S presented the smallest significant difference concerning the control treatment (Figure 1).

Several authors have observed that the growth of different plant species is affected in the same way when they are growing in solutions lacking nutrients (Prado *et al*., 2007; Maia *et al*., 2011) since the nutrients are fundamental for all the metabolic processes and structural formation in plants, as described in Marschner (1995).

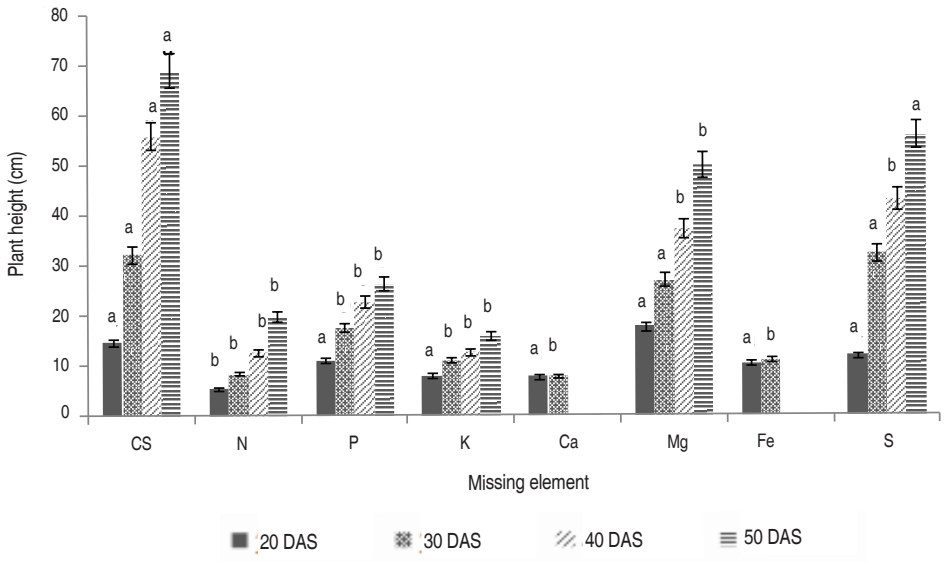

Figure 1. Plant height at 20, 30, 40, and 50 DAS cultivated with complete solution (CS) and with the omission of nutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and sulfur (S). Means (for each date) followed by the same letter do not differ (*P*>0.01).

The omission of nutrients significantly affected the sunflower stem diameter. At 50 DAS, the plants submitted to the omission of N, P and K presented stem diameter 79.4, 73.13 and 69.49% lower than the plants submitted to the complete solution. There was also a significant effect on Ca and Fe omissions, although it was only possible to evaluate these differences up to 30 DAS. In this period, the plants submitted to the solutions with Ca and Fe omission obtained a diameter of 72.06 and 63.51% smaller than that observed in the plants under complete solution. There was no significant effect for treatments containing magnesium and sulfur (Figure 2).

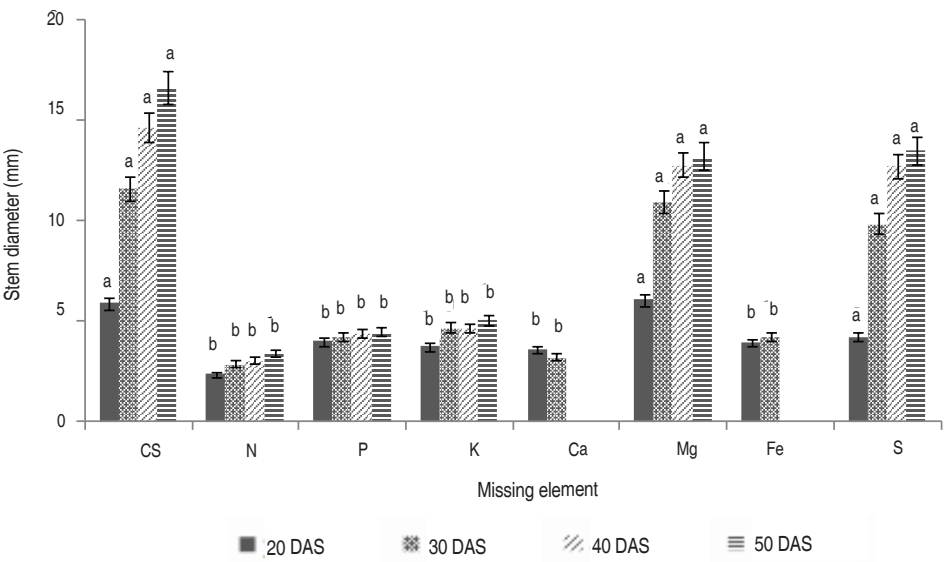

Figure 2. Stem diameter at 20, 30, 40, and 50 DAS cultivated with complete solution (CS) and with the omission of the nutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and sulfur (S). Means (for each date) followed by the same letter do not differ (*P*>0.01).

These results were corroborated by Prado and Leal (2006), Coelho *et al.* (2012), and Gondim *et al*. (2016), who evaluated the stem diameter of sunflower plants, var. Catissol-01, ornamental ginger, and cultivar 1030 maize plants, respectively.

The omission of nutrients, mainly N, P, K, and Ca impacted the stem diameter severely since these are the main responsible for the structural formation of plants (Marschner, 1995). Leaf emission and consequently the sunflower leaf area were strongly affected by the omission of nutrients, with a significant difference when N, P, K, Ca, Fe, and S were omitted (Figure 3).

The absence of these elements in the nutrient solution was responsible for the senescence of the leaves, decreasing their number in the plants. It can be observed in plants submitted to the solution with the omission of

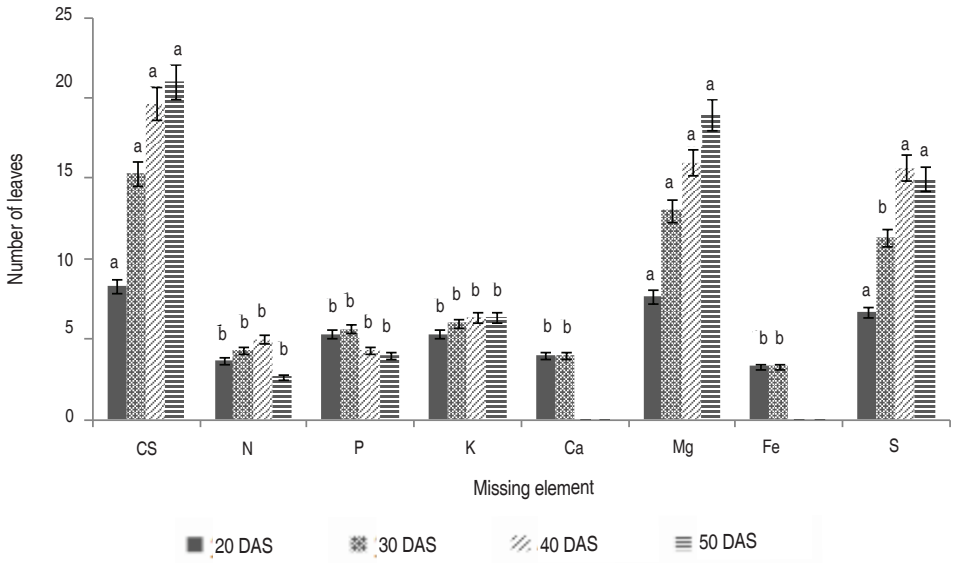

Figure 3. Number of leaves of sunflower plants at 20, 30, 40, and 50 DAS cultivated with complete solution (CS) and with the omission of nutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and sulfur (S). Means (for each date) followed by the same letter do not differ (P>0.01).

N and P which initially had a higher average number of leaves at 20 DAS than the one verified at 50 DAS. At 30 DAS. With Ca, Fe, and S omission there were differences of 73.91, 78.26, and 26.09%, respectively, concerning complete nutrient solution. In the last evaluation, 50 DAS, the differences were 87.3, 80.95, and 9.84%for treatments with the omission of N, P, and K, respectively. The deleterious effects due to the missing elements caused a decrease in the leaf area of sunflower (Figure 4), reducing the surface of light absorption for the photosynthesis as commented by Castro *et al*. (2015).

Probably this reduction occurred due to the low number of leaves, corroborating Maia *et al*. (2014), who stated that the lack of micronutrients in a nutrient solution did not affect the leaf area of plants. The leaf area of plants cultivated with the absence of N, P, K, Ca, and Fe corresponded to 31.89, 43.61, 58.9, 30.22, and 30.57 cm^2, respectively, while the plants that received complete solution reached an average leaf area of 1837.87 cm^2 at 40 DAS.

The omission of N, P, K, Ca, and Fe significantly reduced the dry matter of sunflower plants around 90% in relation to the control (Figure 5A and 5B), corroborating Gondim *et al*. (2016), who observed a reduction in the dry matter of the corn plants, BRS 1030 cultivar, with the deficiency of N, P, K, and Ca in nutritive solution. As previously mentioned, these nutrients are essential for the adequate mineral supply of plants in order to provide normal plant growth. Since there were omissions of these nutrients in the established treatments, the growth of sunflower plants was affected as well as their dry biomass.

Therefore, the omission of N, P, K, Ca, and Fe promoted a restriction on the growth of sunflower plants, with a significant treatment effect on height (Figure 1) stem diameter (Figure 2), number of leaves (Figure 3) and leaf area (Figure 4) of plants.

About the omission of N and K for sunflower lineage LA 1, Cruz *et al.* (1983) observed deficiency symptoms and a significant decrease of the dry matter of the plants relative to the complete nutrient solution. According to Prado and Leal (2006), the individual omissions of N, P, K, and

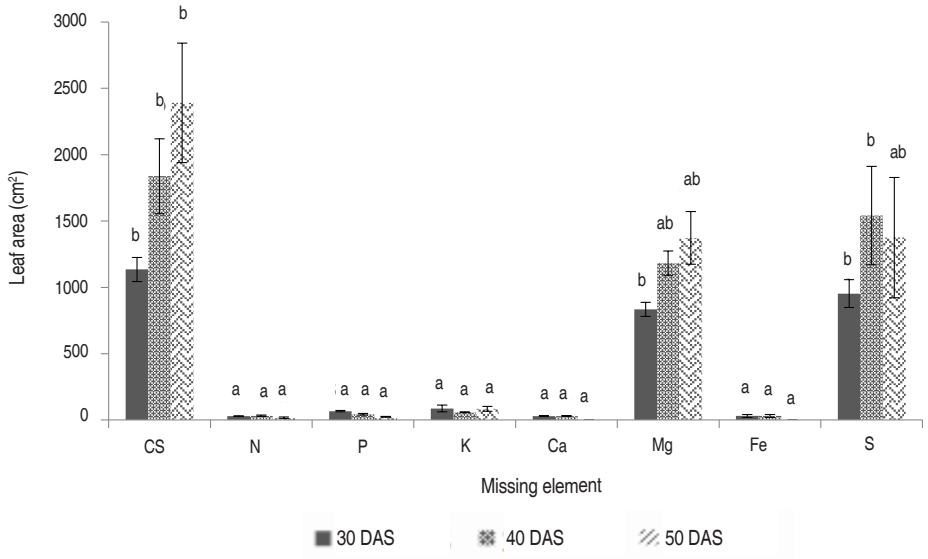

Figure 4. Leaf area of sunflower plants at 20, 30, 40 and 50 DAS cultivated with complete solution (CS) and with the omission of nutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and sulfur (S). Means (for each date) followed by the same letter do not differ (P>0.01).

Ca limited the vegetative growth of the sunflower (cv. Catissol-01) and the dry matter produced by the plants. Concerning sulfur omission, there was no effect on the plant height (Figure 1), stem diameter (Figure 2), number of leaves (Figure 3), and leaf area (Figure 4). On the other hand, S omission affected the dry matter yield of sunflower plants (Figure 5), in relation to the control.

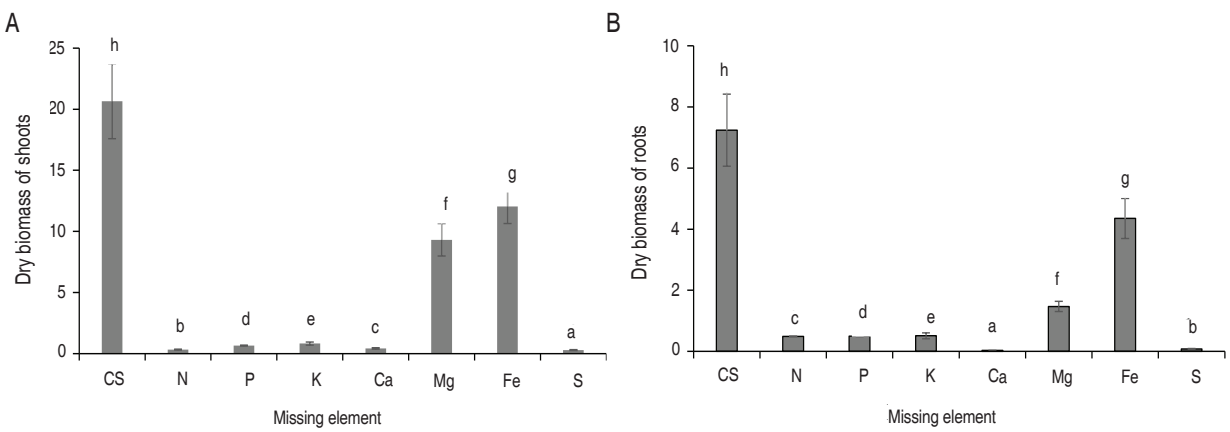

Figure 5. Dry biomass of A. shoots; B. roots of sunflower plants at 50 DAS with complete solution (CS) and with the omission of nutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and sulfur (S). Means (for each date) followed by the same letter do not differ (P>0.01).

Description of visual symptoms

The visual deficiency symptoms were initially recorded from DAS by photographs and daily described throughout the experimental period (50 DAS), they are described below.

The absence of nitrogen in the nutrient solution affected significantly the sunflower plants, this was identified at the beginning of the plant growth (20 DAS), uniform chlorosis of the vegetative part of the older leaves, and then reaching all leaves of the plant (Figure 6B), corroborating the findings of Malavolta (2006). According to this author, the nitrogen deficiency in plants results in the collapse of the chloroplasts, occurring a decline in the levels of chlorophyll. Over time, the older leaves were dried from the tip to the ribs and the intense and uniform yellowing reached the younger leaves (Figure 6B).

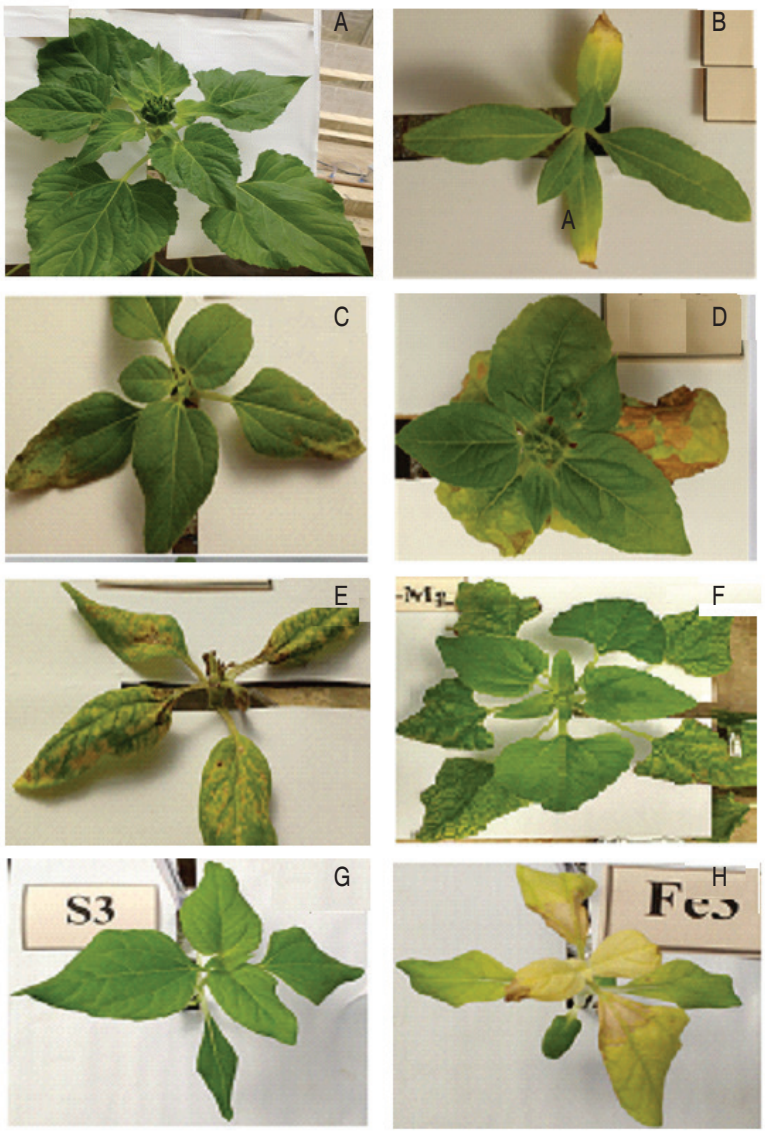

Figure 6. Visual symptoms in sunflower plants cv. BRS-122 cultivated in A. Complete solution; with B. Omission of nitrogen; C. Phosphorus; D. Potassium; E. Calcium; F. Magnesium; G. Sulfur; H. Iron.

According to Epstein and Bloom (2006), the lack of nitrogen causes chlorosis in the leaves, reducing its photosynthetic capacity, the growth rate of the plants, and, in extreme cases, can cause growth paralysis. Therefore, when the N content in the plant is deficient, several physiological processes are compromised and then evolve to visual symptoms of deficiency. Plants under omission of N redistribute via phloem, exhibiting yellow coloration in their older parts (Malavolta *et al*., 1997).

The appearances of brown staining at the edge of the older leaves evolving from the base and from the older leaves to the younger leaves were the visual symptoms of phosphorus deficiency in sunflower plants (Figure 6C). At an advanced stage, the older leaves presented necrosis throughout the leaf edge.

Potassium plays an important role in regulating the osmotic potential of plant cells. It also activates many enzymes involved in respiration and photosynthesis (Taiz and Zeiger, 2009). The symptoms of K deficiency in the sunflower plants were found in older leaves in the form of chlorosis, followed by necrosis of leaf margins and tips. With the intensification of symptoms, it was also observed bending of the youngest leaves (Figure 6D). These symptoms were also observed by Prado and Leal (2006) in research with sunflower.

The Ca omission in the nutrient solution caused chlorosis in the younger leaves, presenting shading in the leaf limbus. Another symptom very characteristic of this element omission is the death of the pointers; and with the continuity of the experiment, the leaves began to show symptoms of necrosis, evolving to the death of the plants. Necrosis in plants can be preceded by generalized chlorosis and bending down the leaves (Figure 6E). Growth can be severely affected if the meristematic regions of the plant die prematurely (Taiz and Zeiger, 2009).

Plants submitted to Mg omission presented visual symptoms of their deficiency: internerval chlorosis, which evolved to bleaching and necrosis of bleached areas, and the old leaves were re-enwrapped and rolled (Figure 6F). Depigmentation is a characteristic symptom of the effects of Mg deficiency since this element is part of the structure of the chlorophyll molecule and its deficiency causes chlorosis (Taiz and Zeiger, 2009). Magnesium is easily redistributed into the plant, so deficiency symptoms usually appear first on older leaves. This pattern of chlorosis occurs because chlorophyll in vascular bundles remains unchanged for more extended periods than chlorophyll in cells between bundles. In severe deficiency, the leaves turn yellowish or white.

The symptoms of sulfur deficiency did not appear at any stage during the 50 experimental days (Figure 6G). However, Malavolta *et al*. (1997) reported that the main characteristic symptom is a yellowing of the younger leaves, which was observed by Cruz *et al*. (1983), cultivating sunflower lineage LA 1 under greenhouse conditions.

The sunflower plants cultivated with nutrient solution without Fe presented reduction in size and chlorosis, initially in the younger leaves (Figure 6H) and then in the medium leaves. Subsequently, the new leaves became completely chlorotic, almost white evolving to necrosis of the leaves (both in the margins as in the surface of the leaves in scattered points). The leaves become chlorotic because iron is required for the synthesis of some chlorophyll-protein complexes in the chloroplast (Taiz and Zeiger, 2009).

CONCLUSIONS

The omission of nitrogen, phosphorus, potassium, calcium, and iron in the nutrient solution severely affected the sunflower plants, preventing their vegetative growth and consequently their development.

The negative interference of the magnesium and sulphur omission in sunflower growth was less harmful than the observed for nitrogen, phosphorus, potassium, and calcium. The absence of nutrients gave clear evidence of the distinct effects that the omission of each element can cause on the visual aspects of sunflower plants.

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