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Tolerance of chia seeds to copper

Tolerância de sementes de chia ao cobre

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ABSTRACT
Environmental contamination by toxic metals has become a problem for plants, animals and man. Among the toxic metals present in the environment, copper (Cu) is the most important contaminant and can, when excess in water or soil, cause disturbances in the growth and development of plants, reducing the productivity of crops. The objective of this study was to evaluate the tolerance of chia seeds to copper during germination. The seeds were placed on paper soaked in aqueous copper solution at concentrations corresponding to zero (distilled water); 60; 120; 180 and 240 mg L^{-1}. The evaluated parameters were: percentage of germination, first count, total length, shoot and root length and dry mass of seedlings. The increase of copper concentration in the substrate promoted a significant decrease in seed germination, growth and dry mass of chia seedlings. It is concluded that chia seeds moderately tolerate exposure to copper at concentrations of up to 120 mg L^{-1} of Cu in the germination phase and up to 60 mg L^{-1} in the initial development phase.
Keywords: germination; *Salvia hispanica*; toxicity.

RESUMO
A contaminação ambiental por metais tóxicos tornou-se um problema para as plantas, animais e para o homem. Dentre os metais tóxicos presentes no ambiente, o cobre (Cu) é o contaminante mais importante, podendo, quando em excesso na água ou no solo, causar distúrbios no crescimento e desenvolvimento das plantas, diminuindo a produtividade das culturas. O objetivo deste estudo foi avaliar a tolerância das sementes de chia ao cobre na germinação. As sementes foram colocadas sobre papel embebido em solução aquosa de cobre nas concentrações correspondentes a 0 (água destilada), 60, 120, 180 e 240 mg L^{-1}. Avaliaram-se os seguintes parâmetros: percentagem de germinação, primeira contagem, comprimento total, da parte aérea e da raiz e massa seca de plântulas. O aumento da concentração de cobre no substrato promoveu decréscimo significativo na germinação das sementes, no crescimento e na massa seca das plântulas de chia. Concluiu-se que as sementes de chia toleram moderadamente a exposição ao cobre nas concentrações de até 120 mg L^{-1} de Cu, na fase de germinação, e até 60 mg L^{-1}, na fase de desenvolvimento inicial.
Palavras-chave: germinação; *Salvia hispanica*; toxicidade.

INTRODUCTION

Agricultural growth has resulted in environmental problems caused by the presence of residues containing toxic portions of copper (Cu). The contamination of soil by this element is a well-known concern, particularly in abandoned agricultural and mining areas (SACRISTÁN *et al*., 2016) where concentrations of copper are often high, due to the various others anthropogenic processes involved such as the application of fungicides and inorganic fertilizers, foundries, mining and atmospheric emissions (HARICHOVÁ *et al*., 2012). In addition, repeated applications of manure, intended to enrich the soil, derived from waste of pigs and poultry which have this element included in their diets to promote growth, can lead to an excess of copper in the land (RUYTERS *et al*., 2013).

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At low concentrations, copper is an essential micronutrient to plants as a constituent and activator of many enzymes. It acts as a cofactor of proteins in the electron transport chain and in mitochondria and chloroplasts. Further, copper participates in photosynthesis, respiration, metabolism of nitrogen and carbohydrates, antioxidant activities, metabolism of cell walls, hormonal assimilation and in the synthesis of lignin and chlorophyll (KARIMI *et al*., 2012).

However, even though it is an essential nutrient, copper can become toxic to plant growth after reaching a certain level. Concentrations above 40 mg Kg^{-1} (sandy soils) and 100 mg Kg^{-1} (clayey soils) can be toxic and negatively affect growth and development (FIDALGO *et al*., 2013).

The toxicity causes many disorders: damages photosynthesis; disrupts the thylakoid membrane integrity resulting in chlorosis and necrosis; reduces root and shoot growth; inhibits the absorption of water; alters the metabolism of nitrogen; and decreases germination (YRUELA, 2009; FIDALGO *et al*., 2013). Kalai *et al*. (2014) state that germination inhibition may occur due to a failure in mobilization of the endosperm caused by the decline of α-amylase, acid phosphatase and alkaline phosphatase activity, as well as a small modification of β-amylase activity, resulting in failure in the mobilization of copper in the endosperm.

Copper can be potentially toxic to plants when it is at elevated concentrations in soil and water (ADREES *et al*., 2015). Thus, phytotoxicity assays are particularly relevant when contaminants are present in soil (KO *et al*., 2012). Some works using copper have demonstrated that, at high concentrations, this element has a negative effect on germination and emergence of various crops: wheat (*Triticum aestivum* L.), white mustard (*Sinapis alba* L.), rapeseed (*Brassica napus oleifera* L.) (DRAB *et al*., 2011); barley (*Hordeum vulgare* L.) (KALAI *et al*., 2014); black oats (*Avena strigosa* Schreb.) (GIROTTO *et al*., 2014); chickpea (*Cicer arietinum* L.) (SMIRI & MISSAOUI, 2014); sunflower (*Helianthus annuus* L.) (BOROŞ & MICLE, 2015); lettuce (*Lactuca sativa* L.) (MORAES *et al*., 2015); grasses (*Brachiaria brizantha* (Trin.) Griseb., *Brachiaria decumbens* (Trin.) Griseb.) (BORGES *et al*., 2016); fenugreek (*Trigonella foenum-graceum* L.) (MENON *et al*., 2016); *Bauhinia forficata* Link, *Enterolobium contortisiliquum* (Vell.) Morong, *Pterogyne nitens* Tul. (SILVA *et al*., 2016) and radish (*Raphanus sativus* L.) (CHITRA, 2017). In general, these authors suggest that an increase in copper concentration in the substrate negatively affects seed germination of the species.

Chia (*Salvia hispanica* L. – Lamiaceae) is a plant grown from seeds (figure 1). Chia seeds have high protein, fiber, oil and fatty acid (omega 3) levels, making them a health-enhancing food, especially regarding their ability to reduce the risk of cardiovascular diseases, maintaining body weight and reducing cholesterol levels in the blood (COELHO & SALAS-MELLADO, 2014). Originally from central-west Mexico to northern Guatemala, it is commercially cultivated in Argentina, Colombia, Peru, Australia, Guatemala and Mexico (BUSILACCHI *et al*., 2013). In Brazil, the regions of northeastern Rio Grande do Sul and western Paraná have begun to commercially invest in this crop, with good results (MIGLIAVACCA *et al*., 2014).

Figure 1 – *Chia* plants in the field.

It is now a plant that has economic and nutritional importance. However, little is known about the behavior of chia in soils with elevated concentrations of toxic elements, specifically development under toxic copper conditions. The information about the tolerance of chia seeds to excess copper on the germination of seeds can contribute to new knowledge about the physiology of this crop and is the objective of this work.

MATERIAL AND METHODS

The experiment was carried out at the Botanic Genetic Laboratory of the Departament of Biology, at the Federal University of Santa Maria, State of Rio Grande do Sul, Brazil, from February 15 to April 27, 2017. The experimental design was completely randomized, where treatments consisted of different concentrations of the solution. This study used chia seeds (*Salvia hispanica* L.) that were acquired from a company that produces and sells seeds in Burzaco, Argentina, and stored in a refrigerated chamber at 10°C until the experiment.

To evaluate the effect of copper on germination, chia seeds were sown on a germitest paper substrate moistened with aqueous solutions of copper sulfate at concentrations of zero (control), 60, 120, 180, and 240 mg L^{-1} (adapted from SILVA *et al.*, 2016 and BORGES *et al.*, 2016). For the control (level zero), only distilled water was used.

The toxic effect of copper on the seed germination process was evaluated using the tests listed below:

Germination: conducted based on four repetitions of 100 seeds distributed in plastic boxes (Gerbox), on germitest paper moistened with distilled water or copper sulfate solution (2.5 times the weight of the paper). After sowing the seeds, the plastic boxes were maintained in BOD (Bio-Oxygen Demand) chambers at a constant temperature of 20°C and 8 h of light and 16 h of dark. Counts were made on seven and 14 days after sowing and the results were expressed as percentages (BRASIL, 2009).

First count: conducted together with the germination test, where the percentage of normal seedlings was determined on day 7 of the test.

Seedlings length (cm): normal seedlings were obtained by sowing four repetitions of 20 seeds. Rolls of paper containing the seeds were kept in a germination chamber for seven days, at a temperature of 20°C. Total length, shoot length and root length of 10 seedlings were randomly evaluated for each repetition using a millimeter ruler. The average length of the seedlings was obtained by adding the number of measurements of each repetition and dividing this by the number of normal seedlings measured (NAKAGAWA, 1999).

Dry mass of seedlings (mg): first, the fresh weight of 10 seedlings was measured (four repetitions), after this, they were placed in paper bags in an oven at 60°C until the mass was constant (48 h). Subsequently, the seedlings were weighed again using a precision scale (0.001 g).

The data were submitted to an analysis of variance using the F test and, when significant, a regression analysis was performed using the program Sisvar (FERREIRA, 2011).

RESULTS AND DISCUSSION

The analysis of variance indicated significant differences ($p<0.05$) among the treatments for all the variables analyzed (table 1).

Table 1 – Summary of the analysis of variance for the variables germination (G), first count (FC), total length (TL), shoot length (SL), root length (RL) and dry mass (DM) of chia seedlings exposed to different concentrations of copper.

Source of variation	Degrees of freedom	Mean square					
		G	FC	TL	SL	RL	DM
Treatment	4	3505.5*	11020.2*	13.23*	0.74*	8.62*	0.097*
Residue	15	274.5	588.7	5.16	0.65	4.18	0.072
CV (%)	–	5.48	12.06	7.62	7.16	11.07	12.76

* Significant at 5% probability by the F test. CV = Coefficient of variation.

In the absence of copper, the average seed germination was 93% (figure 2). The chia seeds were moderately tolerant up to the concentration of 180 mg L^{-1} Cu, with over 70% germination (on average). The highest concentration used (240 mg L^{-1}) promoted a significant reduction (55%).

In the first count germination test, there was a reduction in percentage of normal plants from 88% (control) to 16% (240 mg L^{-1}). The results of this study corroborate Menon *et al*. (2016), who verified that increasing copper concentrations inhibit seed germination and growth of *Trigonella foenum-graceu*, as a notable decrease in germination was observed, starting at 200 ppm of copper.

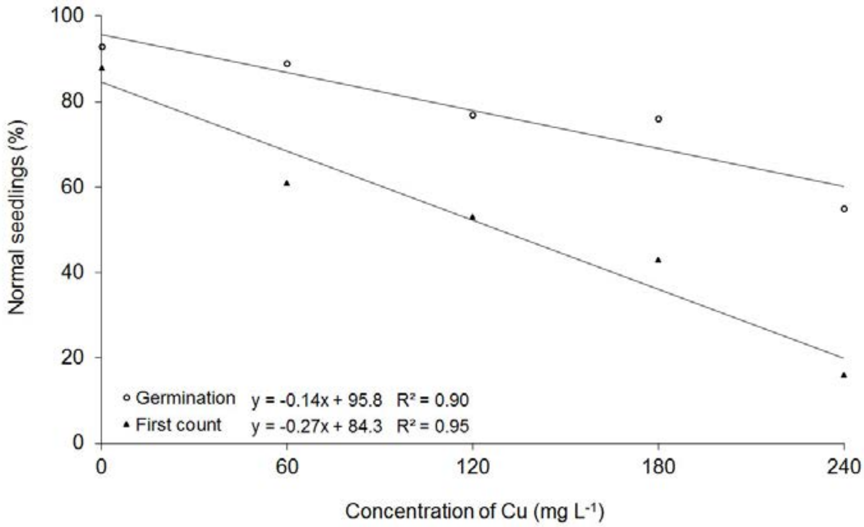

Figure 2 – Germination and first germination count of chia seeds exposed to different concentrations of copper.

The effects of copper on germination are documented in many studies.

Drab *et al*. (2011) observed that copper sulfate caused a significant reduction in seed germination of wheat (*Triticum aestivum* L.), rapeseed (*Brassica napus oleifera* L.) and white mustard (*Sinapis alba* L.), using solutions of 0 to 20 mg dm^{-3}.

Mohammadi *et al*. (2013) analyzed five levels of copper sulfate (0 to 60 mg L^{-1}) and found a reduction in the germination percentage and length of the hypocotyl of *Plantago psyllium* above 45 mg L^{-1}.

Smiri & Missaoui (2014) noticed that this element affected seed germination and growth of chickpea embryos (*Cicer arietinum*), at concentrations of 100 to 500 μM.

Boroş & Micle (2015) observed that, for seeds of sunflower, concentrations of 50 ppm and 100 ppm were more toxic, causing abnormal development and low viability of seeds.

Silva *et al*. (2016) showed that increased doses of copper in soil (0 to 300 mg kg^{-1}) negatively interfered with (at greater intensity) the growth and quality of *Bauhinia forficata*, *Enterolobium contortisiliquum* and *Pterogyne nitens* seedlings.

Chitra (2017) evaluated the response of radishes under the influence of copper (0 to 100 mg L^{-1}) and observed that an increase in the concentration of copper affected the germination percentage, length of the root and of the aerial parts, fresh weight of the root and of the aerial parts, and vigor index.

In relation to total length of the chia seedlings (figure 3A), there was a reduction from 8.41 cm (control) to 7.08 cm (240 mg L^{-1}). Similarly, the length of the aerial part decreased from 3.01 cm (control) to 2.72 cm and the length of the roots decreased from 5.40 cm (control) to 4.36 cm for the highest concentration of copper used (240 mg L^{-1}). The dry mass decreased from 0.65 mg to 0.47 mg (240 mg L^{-1}) (figure 3B).

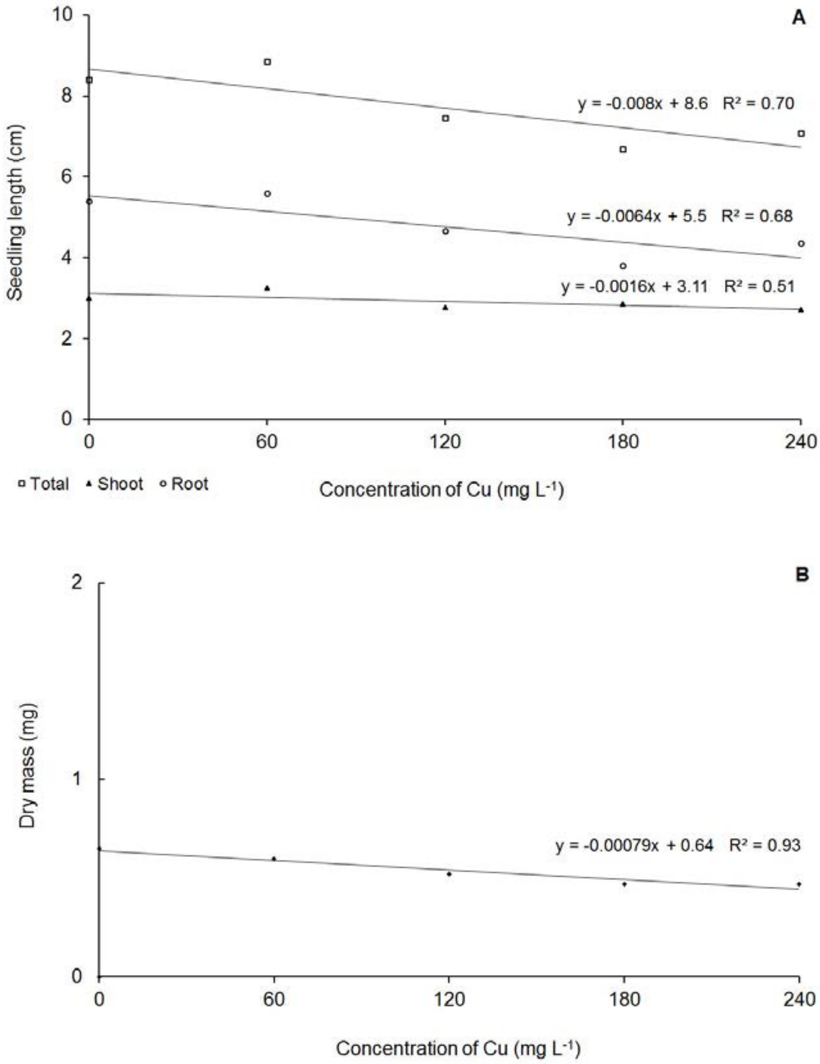

Figure 3 – Length (A) and dry mass (B) of *chia* seedlings exposed to different concentrations of copper.

It is known that plants need low quantities of copper in the soil, and that seeds can germinate under these conditions. However, high quantities of copper in the soil can affect the development of seedlings, ceasing root growth by interfering with mitotic division (YILDIZ *et al*., 2009) or causing a shortage of nutrients for the embryo due to a decrease in the mobilization of reserves in the cotyledons (KARMOUS *et al*., 2011). According to Jones *et al*. (2013), tolerance to toxic metals varies among individuals of the same species and among different species. For example, corn is more sensitive to copper than beans and, when exposed to the same concentration of copper, these species exhibit different toxic effects (KO *et al*., 2012).

Seeds use nutrient reserves as a source of metabolites for respiration. The toxic solution enters the seeds through the teguments and causes oxidative stress and interferes with the activity of enzymes, such as α-amilase e β-amilase, which are responsible for the degradation of starch that is the main resource in seeds used during the germination process (KO *et al*., 2012). Therefore, the inhibition of these enzymes can indicate the mechanisms of toxicity and sensitive plants exposed to these elements can serve as indicators in contaminated environments because they respond rapidly to the deleterious effects (KONG, 2013).

Using other solutions that also simulate copper toxicity, Boroş & Micle (2015) found that the higher the concentration of copper, the more negative the effects were on the length of the aerial part and of the root of sunflowers at concentrations of 50 to 100 ppm. Silva *et al*. (2010) worked with different levels of copper and found a reduction in the growth of *Peltophorum dubium* seedlings at concentrations over 150 mg kg^{-1}.

At 200 mg L^{-1} of copper, Borges *et al*. (2016) observed a high incidence of *Brachiaria brizantha* (cv. Piatã, Marandu e MG5) and *Brachiaria decumbens* seedlings without a radicle. In addition, Girotto *et al*. (2014) observed a reduction in dry mass of the root and aerial parts, as well as interveinal chlorosis, when *Avena strigosa* was cultivated in high concentrations of copper. According to Feng *et al*. (2016), the root is the primary part of the seedling that is in contact with a contaminated substrate and, because of this, the length of the radicle is one of the most affected parameters in tests with this element (LI *et al*., 2007; MUCCIFORA & BELLANI, 2013). This metal can interfere with mitotic division causing the radicle not to grow or reducing its length (LIU *et al*., 2014). The loss of apical dominance is another factor that influences radicular length due to an increase in branching and the formation of edemas in the roots (KOPITTKE *et al*., 2007).

Indications of copper toxicity can vary among plant species. However, generally, there are changes in root growth of cultivated plants in soil with high levels of this element. Among the signals, a dark coloration, thickening, reduction in length, abnormal branching (PAVLÍKOVÁ *et al*., 2007) and thinner roots with dark apices are cited, as observed in the present study.

This work showed that an increase in copper concentration in the substrate promoted a significant decrease in the germination of chia seeds. These results can be used to help in the selection and careful application of fungicides and fertilizers with high concentrations of copper. In addition, a detailed investigation of the soil and water for the presence of toxic metals is recommended before cultivating chia.

CONCLUSION

The chia seeds moderately tolerate exposure to copper, up to 120 mg L^{-1} during the germination phase and up to 60 mg L^{-1} in the initial development phase.

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