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SEED GERMINATION AND SEEDLING GROWTH OF *ZEA MAYS* UNDER VARIOUS CONCENTRATIONS OF COPPER

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Abstract: The objective of this work was to verify the effects of copper on the germination and growth of corn seedlings in substrate of paper for germination plus copper at concentrations of 0, 100, 200 and 300 mg/L, generating four different treatments. The evaluations were carried out on the fourth, fifth, sixth and seventh days after sowing, and copper affected the initial germination rate and root size, without having any influence on shoot size.
Keywords: Corn; heavy metal; phytotoxicity.

INTRODUCTION

Heavy metals comprise around 40 elements, which have a minimum density of 6.0 g cm^{-3} (Alloway, 2013). Many of these metals, such as copper, are essential for the growth of eukaryotic and/or prokaryotic organisms, being required in very small concentrations. However, in high concentrations, they become extremely toxic, affecting animals, plants and microorganisms (Volesky, 1990).

Copper is the twenty-fifth most abundant element in the earth's crust, with atomic number 29 with atomic mass 63.546 (Lee, 1999). Its essentiality as a micronutrient for plants was first established by researchers Lipman and Mcknney in 1931 (Marschner, 2011).

Soils are naturally composed of several elements, including heavy metals, arising from the weathering of the parent material. In soils with anthropogenic influence, the sources of addition of heavy metals are atmospheric emissions from metallurgical industries, fertilizer plants, combustion of coal and fossil fuels, mining and incinerators, in addition to the direct application of biosolids, animal waste, composed of urban waste, fertilizers and agricultural correctives, pesticides and other products used in agriculture (Teicher, 2014). These materials contribute varying amounts to the entry of heavy metals into the environment.

According to data from the Brazilian Institute of Geography and Statistics (IBGE, 2021), in the third quarter of 2021 alone, more than 13.72 million pigs were slaughtered in Santa Catarina. In this state, swine farming is an activity developed on small properties, in which the waste produced is used as fertilizer in areas with annual grain crops and pastures (Girotto et al., 2010). This is desirable, since the nutrients contained in the manure are reused in the production unit itself. However, in many properties the amount of manure produced exceeds the carrying capacity of the soils, and they are applied continuously in the same areas, in frequencies and amounts that are excessive in relation to the absorption capacity of the cultivated plants (Basso, 2003; Berwanger, 2006). As a consequence, due to the high concentration of trace elements such as copper in manure (Gräber et al., 2005), excessive accumulation of this element in the soil is expected over the years (Girotto et al., 2010).

Copper is generally taken up by plants as a divalent cation(Cu^{2+}), but at high pH it can be absorbed as a cation (Cu(OH)2) (Sposito, 2016; Alloway, 2013; Kabata-Pendias, 2011).

The form of translocation from the root to the shoot occurs in the xylem and phloem, where the metal is surrounded by nitrogenous organic compounds, such as the amino acids (Alloway, 2013).

Copper is a micronutrient that acts as a structural element of several proteins, involved in the electron transport chain, in mitochondria and chloroplasts, as well as in the response to oxidative stress in plants (Gratão et al., 2005; Yruela, 2009). It is also involved in cell wall metabolism, hormone signaling, protein metabolism and iron mobilization (Pilon et al., 2006; Yruela, 2005).

It acts as an enzymatic cofactor of several enzymes, also acting, at the molecular level, in transcriptional signaling, oxidative phosphorylation and iron mobilization (Yruela, 2005).

Due to the low requirement of this micronutrient, high doses can cause toxicity to plants (Van Assche and Clijsters, 1990). Toxicity depends on the species and the physiological stage of growth, and in general the symptoms can cause biomass reduction, chlorosis symptoms and affect root development (Yadav et al., 2010).

Excess copper causes alterations in photosynthesis, through disturbances in electron transport, reduction in the number of chloroplasts and damage to the membrane structure of thylakoids (Sandmann and Böger, 1980; Ciscato et al., 1997). It causes oxidative stress in the plant, through the generation of reactive oxygen species (Benavides et al., 2005). It exerts deleterious effects on the structure and functions of roots (Jensen and Aöalsteinsson, 1989; Aöalsteinsson et al., 1997), reducing growth or causing necrosis in absorbent roots (Soares et al., 2000).

It also causes deficiency in photosynthetic parameters, since it leads to a decrease in photosynthetic electron transport mediated by photosystem (FS) II and an increase in mediated by photosystem (FS) I. These alterations in photosynthetic electron transport affect the redox status of the stroma and lead to variations in the levels of stromal components, such as pyridine nucleotides (Dhir et al., 2008; Teicher, 2014).

In maize, symptoms of toxicity can alter root growth of plants grown in soils with a high available copper content. Among these, abnormal branching, thickening, dark coloring and reduced elongation are cited (Sheldon and Menzies, 2005; Koppitke and Menzies, 2006; Pavlíková et al., 2007). Excess copper in the aerial part of plants affects important processes, such as electron transport in photosynthesis (Yruela, 2005), also causing a reduction in chlorophyll content and changes in the structure of chloroplasts and in the composition of thylakoid membranes (Pätsikkä et al, 1998; Quartacci et al., 2000). As a result, there is a reduction in biomass and symptoms of chlorosis (Bernal et al., 2006). Furthermore, excess copper induces a reduction in the concentration of iron in the shoot, with consequent interveinal chlorosis in wheat (Michaud et al., 2008) and corn (McBride, 2001) plants.

In Santa Catarina, in the 2022 harvest, more than 339 thousand hectares were cultivated with corn (IBGE, 2022). In general, the world demand for corn has been increasing in recent years, especially with the war between Russia and Ukraine and the demands of Asian countries and the United States. Domestic consumption has also increased considerably as a result of the growth of the meat sector, more specifically poultry and pork. Pavão and Ferreira Filho (2011) explain that the importance of corn in Brazil is wide, firstly because its production occurs both in small properties, whose purpose is subsistence, and in large extensions of land to supply the market; second, because its nutritional importance makes it widely used, not only in human food, but mainly in animal feed.

There are few studies in the literature on the effects of nutritional disorder related to copper toxicity in corn seedlings. Given this context and the importance of the culture, the objective was to verify the effects of toxic and subtoxic concentrations of copper on the germination and growth of corn seedlings grown in a germination chamber.

MATERIAL AND METHODS

Seeds of the simple hybrid corn variety LG 6033, obtained from the Federal University of Fronteira Sul – Campus Chapecó/SC, were used.

The experiment was carried out in the seeds and grains laboratory of the Federal University of Fronteira Sul, Chapecó campus. The trial was planned under a split-plot scheme in time in a completely randomized design, with cross classification and 4 replications (Ares and Granato, 2014; Pimentel-Gomes, 2007). To this end, the seeds were divided into 4 lots of 16 repetitions with 50 seeds and each repetition was sown in a paper substrate for seed germination (J. Prolab®) previously moistened with a volume of water corresponding to 2.5 times the weight of paper. The water used for imbibition of the 4 batches was added with different concentrations of copper sulfate (CuSO4), so that the final concentrations of copper were 0, 100, 200 and 300 mg/L, generating four different treatments. The paper rolls were kept in germinators (ELETROlab®, model 202/4) at a constant temperature of 25ºC, with a constant photoperiod of 24 hours, and the evaluations were carried out in 4 repetitions of each treatment on the fourth, fifth, sixth and seventh days after sowing (Brazil, 2009).

The evaluation of viable seedlings was performed on the fourth, fifth, sixth and seventh day after sowing, in this evaluation abnormal seedlings, hard and dead seeds were quantified, and the data converted to percentage of normal seedlings (Brasil, 2009; Sá et al. al, 2011).

Growth evaluation was performed on the fourth, fifth, sixth and seventh days after sowing, eliminating abnormal seedlings and dead seeds. With the aid of a millimeter ruler, the length of the primary root and length of the shoot were measured and the average results were expressed in cm seedling-1 (Brasil, 2009; Sá et al, 2011).

Analysis of variance (F test at 5%) was used to analyze the variables. Comparisons of the mean of each repetition were made using Tukey's multiple comparisons test or regression, both at 5% confidence (Pimentel-Gomes, 2007; Zimmermann, 2004).

RESULTS AND DISCUSSION

According to the variance test (F test), there is a significant interaction between the days and copper doses factors in relation to the corn germination rate variable (Table 1), indicating the existence of dependence between the factors.

Through the unfolding of the interaction effect, by carrying out a new analysis of variance (F test), in which the levels of the copper doses factor were compared within the levels of the days factor (and vice versa), it was possible to observe that there are significant effects between the time of evaluation within each dose of copper, and the comparison of means between the levels of this factor is represented in table 1.

According to the same test, there are no significant effects between copper doses within each evaluation period.

evaluation period	mg/L de Cobre			
	0	100	200	300
BEDROOM	96,0 a	97,0 a	75,5 a	76,5 a
FIFTH	95,0 a	83,7 a	68,0 a	45,0 b
SIXTH	78,5 a	17,50b	8,5 b	5,0 c
SEVENTH	79,5 a	9,00 b	12,5 b	0,0 c
CV (A) = 18,4% CV (B) = 22,5 %				

Means followed by the same letter do not diverge from each other, in the same column, at 5% confidence.

Table 1. Evaluation of the germination of corn LG 6033 presented in % of normal seedlings

Luchese et al (2004) had already demonstrated that the application of copper in corn seeds at doses of 1 to 6g kg-1 decrease the seed emergence capacity, but without affecting the dry weight of the seedlings that emerged.

Melania-Nicoleta and Micle (2015), using copper concentrations between 0 and 100 mg/L in germination tests similar to those of this experiment, observed that after 7 days the germination rate increased with 10 mg/L of copper, falling 50 % with 50 mg/L and zero with 100 mg/L.

Bashmakov et al., (2005) observed an increase in the germination rate, in relation to the control, at seven days when they submitted corn seeds to a concentration of 63.54 mg/L of copper. When they increased the concentrations to above 63.54 mg/L, they observed a sharp drop in the germination rate.

According to Alaqui-Sossé et al. (2004), excess copper inhibits cell elongation, a complex process dependent on cell turgor pressure, synthesis of wall components and growth regulators, strongly affecting germination.

According to the variance test (F test) there is a significant interaction between the factors of evaluation time and copper doses in relation to the root length variable (Table 2) indicating the existence of dependence between the factors.

Through the unfolding of the interaction effect, by carrying out a new analysis of variance (F test) in which the levels of the copper doses factor were compared within the levels of the evaluation time factor (and vice versa) it was possible to observe a significant effect for the evaluation time factor within each copper dose at the 200 and 300 mg/L levels, and the comparison of means between the levels of this factor is represented in table 2. It was also possible to observe a significant effect for the copper doses factor within each evaluation period, that is, on the fifth, sixth and seventh days, and this effect can be observed through the estimates of the 2nd degree polynomial equations presented in figure 1.

evaluation period	mg/L de Cobre			
	0	100	200	300
BEDROOM	7,1	8,4	12,4 a	11,1 a
FIFTH	5,1	9,8	10,7 a	10,5 a
SIXTH	4,8	5,4	4,9 b	7,4 a
SEVENTH	4,6	4,3	3,6 b	0,0 b
CV (A) = 11,9% CV (B) = 11,5%				

Means followed by the same letter do not diverge from each other, in the same column, at 5% confidence.

Table 2. Length (cm root-1) of roots plantula-1 measured during germination tests.

For copper, the quadratic behavior of root length demonstrates its role in corn physiology as a growth promoter (Table 3), perhaps evidencing its participation as an enzyme activator (Yruela, 2005). It is verified that on the fifth day the length of the roots increased up to 74.4% in relation to the control, when added up to 300 mg/L of copper. However, on the seventh day, the dose of 300 mg/L of copper caused the greatest slope of the germination % curve (21.14% in relation to the dose of 100 mg/L). Copper can be considered toxic at this concentration, as the US Environmental Protection Agency (USEPA) considers the metal content that causes a 50% reduction in growth to be toxic (Humphreys, 2021). In Europe, this rate drops to 25% (Saefl, 1998).

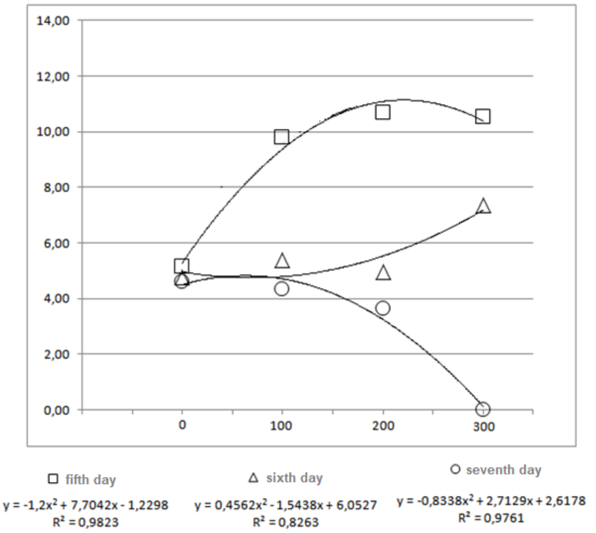

Figure 1. Second degree polynomial equations regarding the effect of copper doses on average root growth (cm seedling $^{-1}$).

This quadratic effect had already been observed by Barbosa et al. (2013), who tested the foliar application of different doses of copper against the parameters leaf area, chlorophyll content, leaf diameter, thousand grain weights and yield. The authors concluded that at lower doses copper promotes an increase in growth and, consequently, an increase in yield, while at high doses it becomes toxic to the crop.

Melania-Nicoleta and Micle (2015) observed an increase in root length at copper concentrations up to 10 mg/L. Concentrations around 50 mg/L caused serious stunting and malformation problems.

According to the variance test (F test) there is no significant interaction between the days and copper dose factors in relation to the shoot length variable (Table 3), indicating that there is no dependence between the factors.

However, it was possible to observe a significant effect for the factors of copper doses and days alone, demonstrating that the effects between these factors occur independently. The comparison of means between the levels of the days factor is represented in Table 4, while the comparison of means between the levels of the copper doses factor is represented through the estimation of the linear equation presented in figure 2.

Excess copper is known to cause reduced shoot growth, as it causes disturbances in electron transport, reduction in the number of chloroplasts, and damage to the membrane structure of thylakoids (Sandmann and Böger, 1980; Ciscato et al, 1997).

evaluation period	mg/L of copper			
	0	100	200	300
BEDROOM	1,84	3,79	3,91	3,58
FIFTH	2,54	3,80	5,12	5,56
SIXTH	1,74	3,01	2,39	1,95
SEVENTH	0,94	0,38	1,24	0,00
CV (A) = 13,2 % CV (B) = 14,3 %				

Table 3. Length of the aerial part of corn seedlings (cm seedling $^{-1}$).

Days	cm Seedling $^{-1}$
BEDROOM	3,2815 ab
FIFTH	4,2525 a
SIXTH	2,2731 bc
SEVENTH	0,6398 c

Means followed by the same letter do not diverge from each other, in the same column, at 1% confidence.

Table 4. Average aerial part size (cm seedling $^{-1}$) measured during germination tests.

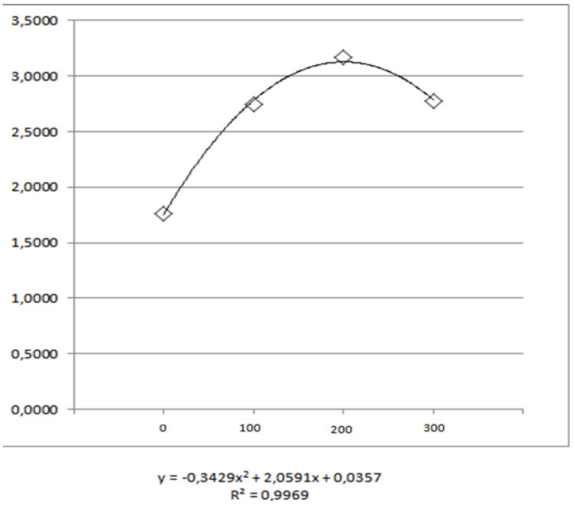

Figure 2. Second degree polynomial equation regarding the effect of copper doses on average shoot growth (cm seedling $^{-1}$).

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In addition, high concentrations of copper inhibit enzymes such as amylases, responsible for degrading the starch reserves in the seed endosperm (Das-Gupta and Mukherji, 1977), impairing seedling growth.

Several more in-depth research is needed to obtain a final result of what is the amount of swine manure recommended for use in areas cultivated with corn that is beneficial for the soil and the crop.

Seganfredo (1999) demonstrated that it is essential to consider that the soil/plant system is incapable of recycling swine manure, while the imbalance between its chemical composition and the amounts of nutrients required by the plants persists.

Regardless of the type of soil and region (Fageria, 1989), the starting point for making agricultural systems fertilized with swine manure self-sustainable is to reduce their pollutant load, highlighting the amount of organic matter and nutrients.

CONCLUSION

A significant interaction was observed between the factors of evaluation time and copper dose in relation to the variable germination rate, indicating that there is dependence between the factors. The effect of copper doses on the germination rate over the evaluated days was differentiated for each dose, with no differences between the doses evaluated for this parameter.

A significant interaction was observed between the factors of evaluation time and copper doses in relation to the variable length of the roots, indicating that there is dependence between the factors.

The effect of the evaluation period factor within each copper dose at the 200 and 300 mg/L levels was evidenced, and it was also possible to observe a significant effect for the copper dose factor within each evaluation period, that is, in the fifth, sixth and seventh days.

No significant interaction was observed between the time of evaluation and copper dose in relation to the shoot size variable, indicating that there is no dependence between the factors. However, it was possible to observe a significant effect for the factors copper doses and evaluation time alone, demonstrating that the effects between these factors occur independently.

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