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Arbuscular mycorrhizal fungi in wheat grown in copper contaminated soil

Fungos micorrízicos arbusculares no cultivo de trigo em solo contaminado com cobre

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Highlights

Wheat development is influenced by high doses of copper in the soil.
Mycorrhizal colonization provides greater root height and length for wheat.
Mycorrhizal fungi allow less copper mobilization for the wheat shoots and grains.

Abstract

At high soil concentrations, copper (Cu) is toxic to plant development. Symbiosis carried out between microorganisms and plant species are alternatives to minimize plant toxicity in copper contaminated soil. The present study aimed to select species of arbuscular mycorrhizal fungi for the development of wheat plants in copper contaminated soil. The experimental design was completely randomized in a 4 × 6 factorial arrangement, with four sources of inocula: three species of arbuscular mycorrhizae (*Acaulospora colombiana, Gigaspora Margarita* and *Rhizophagus clarus*) and one without inoculum (control), and six doses of copper in the soil (0, 80, 160, 240, 320, and 400 mg kg^{-1}), with seven replications. Plant height, root length, root volume, root dry mass, specific surface area, average root diameter, root, shoot and grain Cu content and mycorrhizal colonization rate were evaluated. Root dry mass and mycorrhizal colonization were negatively affected by high Cu doses in the soil. The mycorrhizal isolates *Acaulospora Colombiana* and *Gigaspora Margarita* provide greater wheat height, root length and specific surface area and lower

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Cu content. Mycorrhizal colonization with the fungi *Acaulospora Colombiana, Gigaspora Margarita* and *Rhizophagus clarus* results in lower copper content in wheat shoots and grains.
Key words: Arbuscular Mycorrhizae. Heavy metals. *Triticum aestivum.*

Resumo

Em elevadas concentrações no solo, o cobre (Cu) é tóxico para o desenvolvimento dos vegetais. Simbioses realizadas entre microrganismos e espécies vegetais são alternativas para minimizar a toxidez das plantas em solo contaminado com cobre. O trabalho teve como objetivo selecionar espécies de fungos micorrízicos arbusculares para o desenvolvimento de plantas de trigo em solo contaminado com cobre. O delineamento experimental foi inteiramente casualizado em arranjo fatorial 4 × 6, sendo quatro fontes de inóculos: três espécies de micorrizas arbusculares (*Acaulospora colombiana, Gigaspora margarita* e *Rhizophagus clarus*) e sem inóculo (testemunha) e seis doses de cobre no solo (0, 80, 160, 240, 320 e 400 mg kg^{-1}), com sete repetições. Avaliou-se a altura de plantas, comprimento de raiz, volume de raízes, massa seca de raiz, área superficial específica, diâmetro médio de raiz, teor de cobre na raiz, na parte aérea e no grão e porcentagem de colonização micorrízica. A massa seca de raiz e a colonização micorrízica foram afetadas negativamente com altas doses de Cu no solo. Os isolados micorrízicos *Acaulospora Colombiana* e *Gigaspora Margarita* proporcionam maior altura, comprimento de raiz e área superficial específica e menor teor de Cu no grão na cultura do trigo. A colonização micorrízica com os fungos *Acaulospora Colombiana, Gigaspora Margarita* e *Rhizophagus clarus* resulta em menor teor de cobre na parte aérea e nos grãos do trigo..
Palavras-chave: Micorrizas Arbusculares. Metal pesado. *Triticum aestivum.*

Introduction

Wheat (*Triticum aestivum* L.) is one of the most widely produced cereals in the world (Beltrano et al., 2006). It is a staple food in several countries and one of the main Brazilian raw materials, with a high financial return from its cultivation and the industrial products made from this grain (Farias et al., 2017). Liquid swine manure is a source of organic fertilizer that has been used in wheat crops as a low-cost alternative (Sartor et al., 2012). However, successive applications can lead to nutrient accumulation, including copper, which may cause chemical changes in the soil, toxicity and nutrient imbalance in crops (Magalhães & Weber, 2021).

Cu is an essential micronutrient for living organisms, since it acts in respiration, photosynthesis and nitrogen fixation (Magalhães & Weber, 2021). However, the excessive use of industrial and urban waste, fertilization with animal waste and agrochemicals in agriculture (Andreazza et al., 2013) have led to the accumulation of this element in the soil, causing morphological and physiological changes in vegetables, thereby reducing crop productivity (Magalhães & Weber, 2021). Thus, ordinance 85/2014 established the reference value and soil quality of agricultural areas, derived from volcanic rocks on the State of Rio Grande do Sul plateau, at 203 mg kg^{-1} of total Cu. (Fundação Estadual de Meio Ambiente do Rio Grande do

Sul [FEPAM], 2014), requiring alternatives to keep crops viable in areas contaminated with this element.

Arbuscular mycorrhizal fungi (AMF) may be an alternative for cultivation in copper contaminated soil because they establish a mutualistic symbiotic relationship with around 80% of plant species (Smith & Read, 2008), contributing to development and yield and providing protection from environmental stress and pathogens due to the accumulation of bioactive compounds during growth (Matsubara et al., 2009). These fungi protect plants from the stress caused by high metal concentrations in the soil by maintaining the integrity of cell membranes, promoting metal accumulation in non-vital organs and heavy metal retention by the fungal mycelium or cell walls, with subsequent fixation by cellular polyphosphate granules. (Vendruscolo et al., 2018). However, studies on the cultivation of mycorrhizal wheat in soil containing high Cu levels are still incipient.

Several studies have been carried out with AMF that question the specificity of both the fungus and host species and their symbiotic relationship in environments with excess heavy metals in the soil (Hassan et al., 2013; Santana et al., 2015). As such, efficient AMF species selection is necessary to promote wheat development and maximize the positive effects of this association. The present study aimed to select arbuscular mycorrhizal fungi species for wheat development in cu-contaminated soil.

Material and Methods

The experiment was conducted in a greenhouse at the Forest Engineering Department of the Federal University of Santa Maria (UFSM), Frederico Westphalen Campus, between June and November 2020. The soil used in the experiment was Red Latosol, collected in an agricultural area in the 0 – 20 cm layer and sterilized in an autoclave at 1 Atm (121 °C) pressure for 30 minutes with the process repeated three times. Soil chemical and physical characterization was carried out according to the methodologies described by Mann & Ritchie (1993) for exchangeable Cu and F. C. Silva (2009) for the remaining properties (Table 1).

Table 1
Clay, pH and chemical composition of the soil used for wheat cultivation

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Clay	pH_{water}	MO	P	K	Mg	Al+H	Cu	Zn
%	1:1	%	-------------	-------------	--mg/dm^3--	-------------	-------------	-------------
52.5	5.2	0.9	0.85	24.97	1.92	1.85	8.1	0.59

After soil analysis, the pH of water was corrected to 6.5 and wheat crop fertilization was conducted according to Liming and Fertilization Manual recommendations (Comissão de Química e Fertilidade do Solo, 2016) for an expected yield of four metric tons per hectare. Sixty days before wheat sowing, soil was contaminated with Cu 60 in the form of copper sulfate solution ($CuSO_4.5H_2O$), homogenized in the soil by agitation in a plastic bag.

Arbuscular mycorrhizal fungi (AMF) were obtained from the international collection of glomeromycota culture (CICG) in the mycorrhizal laboratory of the Regional University of Blumenau, Santa Catarina State. Inoculation was performed at sowing, where 30 spores of each mycorrhizal isolate were added per pot. The wheat cultivar used was TBIO Sinuelo. Plastic 5-liter pots filled with 5 kg of soil were used for plant cultivation. Sowing consisted of 10 seeds per pot, carried out in the second half of May, and thinning was performed 15 days after germination, leaving four plants per pot (experimental unit - EU) until the end of the experiment, which was the end of the crop production cycle. The results were based on the average of the four plants for each experimental unit. Irrigation was controlled daily by an automatic drip system, maintaining field capacity at 80%.

The experimental design was completely randomized in a factorial arrangement (4x6), with four sources of inoculum: four mycorrhizal inocula (*Acaulospora colombiana, Gigaspora margarita, Rhizophagus* clarus and control, without inoculum), six doses of copper in the soil (0, 80, 160, 240, 320 and 400 mg kg^{-1} of soil), and seven replications. At the end of the experiment, plant height (PH) was evaluated with the aid of a graduated ruler, measured from the ground to the tip of the spike; root length, estimated using the sapphire program; root volume (RV) measured with the aid of a graduated cylinder; and root dry mass (RDM). The roots were separated from the shoot in the plant base region. RDM was obtained by oven-drying at 61 °C until constant mass and weighed on an analytical balance. The grains were threshed and dried to determine Cu content. After the plants were collected, the roots were separated by washing with water and 0.5 mesh sieves were used to determine the specific surface area (SSA) of the roots according to Tennant (1975). The roots were digitized and the mean root diameter (MRD), root length (RL) and specific surface area (SSA) determined using the SAFIRA program (Jorge & Rodrigues, 2008).

Cu content in shoot, root and grain dry mass was quantified after grinding the samples in a Wiley mill with a 10-mesh sieve. Extraction was via nitro-perchloric digestion (3:1) and copper content was determined using atomic absorption spectrophotometry (Miyazawa, 2009). Soil pseudo-total Cu content was determined according to methodology 3050b described by United States Environmental Protection Agency [USEPA] (1996) and copper extracted with Melhlich-1 solution, which acts by acid dissolution, using sulfuric and hydrochloric acids (Sobral et al., 2013).

The mycorrhizal colonization rate was determined after staining roots with 0.05% trypan blue, as described by Phillips and Hayman (1970), in order to visualize AMF colonization under a stereomicroscope at 60x magnification. (Brundrett et al., 1996). Colonization was estimated at five replicates per plant, totaling 35 replicates per treatment, according to the gridline intersect method (Brundrett et al., 1996).

The results were submitted to analysis of variance (ANOVA) and when there was a significant interaction, the dose effect in each inoculation treatment was revealed by regression analysis. When not significant, the means of the qualitative factor were compared by the Scott-Knot test and the quantitative factor by regression, considering 95% significance levels , using the SISVAR program (Ferreira, 2011).

Results and Discussion

The available and pseudo-total Cu in the soil increased linearly with the application of copper doses (Figure 1). The 95 mg kg^{-1} dose of Cu reaches the maximum (pseudo-total) investigation limit stipulated by Resolution No. 420, which is 200 mg kg^{-1} of soil (Conselho Nacional do Meio Ambiente [CONAMA], 2009). Latosols have a high total metal content due to their parent material, but these metals are predominantly present in unavailable oxide forms, often resulting in plant deficiency (Secco et al., 2005).

The available Cu content in the control group was 8.1 mg kg^{-1} of soil, well below the pseudo-total of 113.7 mg kg^{-1} (Figure 1). The difference in available and pseudo-total Cu content is due to adsorption mechanisms, whereby non-specific adsorption occurs through the action of electrostatic forces between metallic cations in the soil solution and the negatively charged surface of the colloids, making it the most available chemical element. Specific adsorption is due to the high affinity between the colloid surface and free cations in solution, whereby ions penetrate the atom structure and bind to the O- and OH groups on the surface of soil particles through covalent or ionic bonds (Vinhal-Freitas et al., 2010; Araújo, 2015).

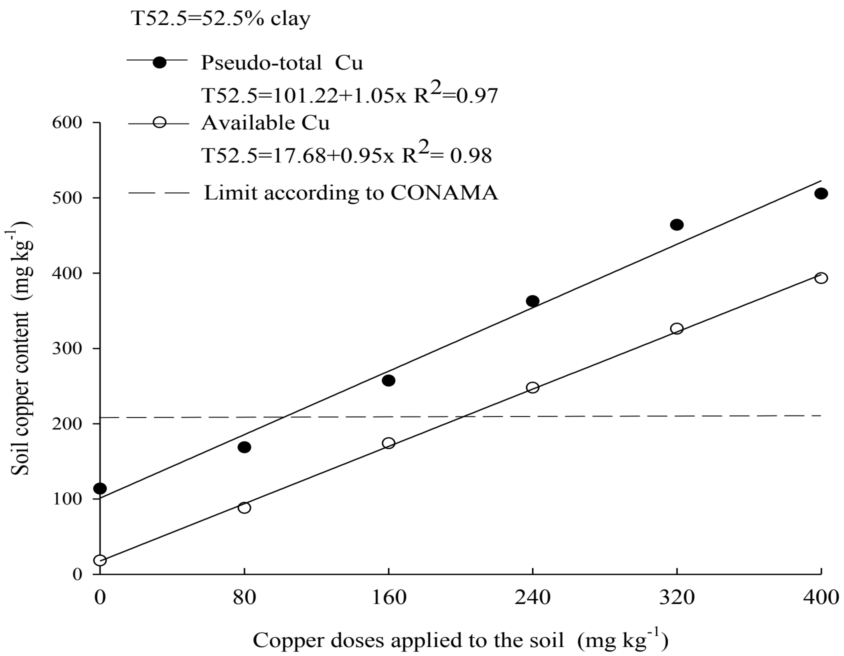

Figure 1. Available and pseudo-total copper in soil cultivated with wheat under increasing doses of 0, 80, 160, 240, 320 and 400 mg of copper kg^{-1} of soil.

The results showed no significant interaction for height, root length, dry mass and root volume, specific surface area and average wheat root diameter, exhibiting only a simple effect for copper doses (Figure 2) and mycorrhizal inocula. (Figure 3).

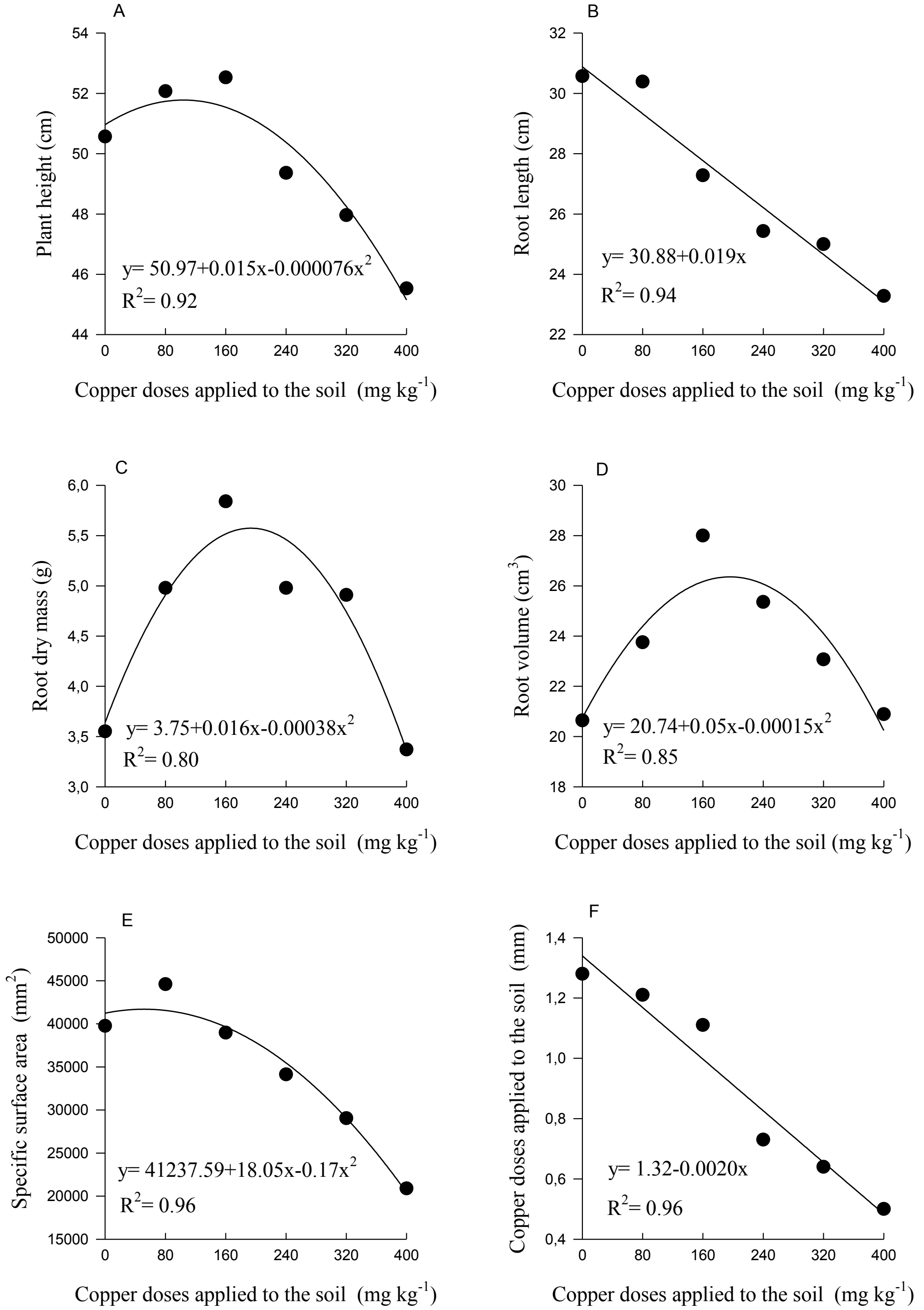

Figure 2. Significant single effect of copper doses for height (A), length (B), dry mass (C), volume (D), specific surface area (E) and average root diameter (F) of wheat plants.

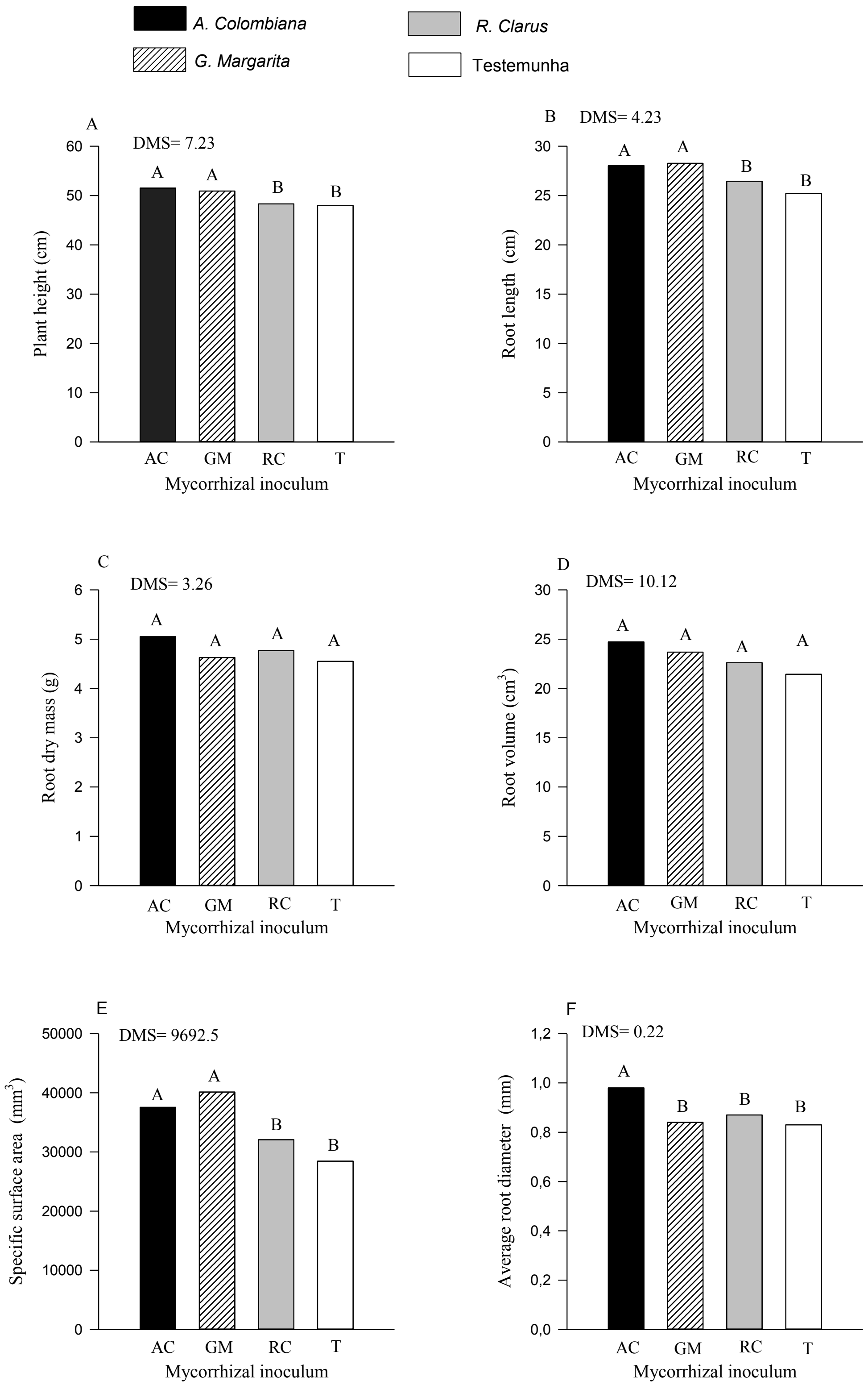

Figure 3. Significant single effect of mycorrhizal inoculum (*A. colombiana, G. margarita, R. clarus* and control without inoculum) for height (A), length (B), dry mass (C), volume (D), surface area specificity (E) and average root diameter (F) of wheat plants.

Cu doses induced a positive quadratic effect on the height of wheat plants, with a maximum effect at 98 mg kg^{-1} of copper in the soil and height of 51.7 cm (Figure 2 A). Micronutrients such as Cu, although required in small amounts, are essential for plants to complete their growth cycle, playing an important role in plant biochemistry and physiology, since their deficiency causes a reduction in yield (Malavolta, 2006). However, reduced plant growth due to high soil Cu levels is reported by Zortéa et al. (2016). In general, wheat plant height declines with the application of 98 mg of Cu kg^{-1} of soil, showing the potential for phytotoxicity.

Increasing Cu doses induced a linear reduction in root length, 23% lower when compared to the application of 400 mg of copper kg^{-1} of soil with the zero dose (Figure 2 B). CU is toxic at high concentrations, reducing root development (Rossi, Amarante, & Fleig, 2008). The root system is one of the main elements that influence the productive capacity of plants, since it plays a key role in crop establishment in the field, being responsible for plant support, water and nutrient absorption efficiency, including phosphorus (P), which displays low soil mobility and whose absorption is directly related to greater root length (Reinert et al., 2008; Pimentel, 2016).

Cu doses quadratically increased dry mass and root volume, reaching a maximum value at 66 mg kg^{-1} with 5.8 grams (Figure 2 C), and at 210 mg kg^{-1} of copper with 26 cm^3 (Figure 2 D). Low soil Cu levels may increase the number of secondary roots and the dry mass of the root system; however, its toxic effect at high doses result in a linear decline in root volume and dry mass (Rossi et al., 2008). This finding is reported in the literature as the absence of root formation, reduced growth and consequent decrease in root mass, reducing the ability to absorb water and nutrients for plant growth and development (Taiz & Zeiger, 2017).

The specific surface area exhibited a quadratic increase with Cu doses and reached a maximum value at 53 mg of copper kg^{-1} of soil (Figure 2 E), and the average root diameter decreased with an increase in the copper dose (Figure 2 F). The specific surface area and average diameter of the root system are related to the ability of plants to absorb water and nutrients from the soil (Taiz & Zeiger, 2017). This is because fine roots are better able to absorb nutrients by diffusion (Novais et al., 2017). As such, Cu in the soil induced a lower specific surface area, length and root diameter in wheat, indicating a reduction and thickening of the root system and, consequently, lower water and nutrient absorption capacity.

The inoculum effect revealed that plant height was higher when the *Acaulospora Colombiana* and *Gigaspora Margarita* AMF species were used for treatment, while *Rizophagus Clarus* did not differ from the control group (Figure 3 A). The association between plants and AMF increases plant metabolism and carbon fixation (Braghirolli et al., 2012), resulting in greater plant height and shoot dry mass (Kumar et al., 2015).

The arbuscular mycorrhizal fungi *G. Margarita* and *A. Colombiana* induced greater wheat root length (Figure 3 B). The hyphae of AMFs penetrate the roots, functioning as an additional root system, increasing root area and, consequently, nutrient absorption capacity (Moreira & Siqueira, 2006). The most noteworthy benefits of mycorrhiza are the extension of the root system, large specific surface area and greater root diameter (Smith & Read, 2008; Costa & Melloni, 2019). Thus, wheat is benefited by mycorrhizal associations due to the increase in volume exploited by the root system.

Root dry mass and volume did not show a significant inoculum effect (Figures 3 C and 3 D). This result corroborates those of Sala et al. (2007), who studied the interaction between AMFs and diazotrophic bacteria in wheat and determined that the isolated inoculation of *Glomus Etunicatun* and *Acaulospora colombiana* did not differ from the treatments without inoculation for the root dry mass of wheat. However, *Rhizophagus Clarus* and *Gigaspora Margarita* inoculation caused an increase in dry mass and root volume in maize (Zea mays) when compared to the control without inoculation (Nunes et al., 2019). This demonstrates the need to select AMF species based on these morphological traits.

The specific surface area of wheat was higher with inoculations of *G. Margarita* and *A. Colombiana* (Figure 3 E). The symbiosis established between arbuscular mycorrhizae and plant species is significant because AMF promote root elongation through their extraradicular hyphae, increasing the contact area in the soil, which can increase the specific surface area up to 40-fold (Giovannetti et al., 2001). As such, several benefits related to the greater specific surface area of plants through symbiosis with AMF are cited in the literature, including increased water and nutrient absorption with low soil mobility (Silveira et al., 2002), as well as resistance to biotic and abiotic stresses (Nunes et al., 2019).

The average root diameter was higher for *A. Colombiana*, but the other mycorrhizal inocula and the control did not differ (Figure 3 F). Root geometry is important for the absorption of nutrients in the soil solution or in the root growth pathway, being absorbed mainly by diffusion and mass flow (Rosolem et al., 2003). Thinner roots exhibit a more favorable geometry for nutrient absorption by diffusion, while thicker roots favor mass flow transport (Taiz & Zaiger, 2017). The inoculation of AMFs in tree species results in a larger root diameter when compared to other treatments (Vandresen et al., 2007).

Root, shoot and grain copper content of wheat plants displayed a significant interaction for doses and mycorrhizal inocula (Figure 4). Root Cu content increased linearly in all treatments, being higher for G. Margarita, with 443 mg kg^{-1} of Cu at 400 mg kg^{-1} (Figure 4 A). In maize, an increase in soil Cu content results in a higher root metal content, with 445.8 mg kg^{-1} at 300 mg dm^{-3} of copper (Seidel et al., 2009). The increase in root metal content and stress relief in mycorrhizal plants, at high concentrations of these elements was reported by Silva et al. (2006). This effect is related to metal adsorption on the hyphae, given that chitin has an important capacity to bind to these elements (Joner et al., 2000). Thus, metal immobilization in fungal biomass is proposed as a mechanism for plant tolerance to heavy metals.

Shoot Cu content increased linearly for the treatment without inoculation and with isolates *A. Colombiana* and *R. Clarus,* being significantly higher at 400 mg kg^{-1} of Cu (Figure 4 B). In the treatment with *G. Margarita,* there was a quadratic response, with a maximum value at 264 mg kg^{-1} of Cu in the soil. Plants associated with mycorrhizal fungi can retain these metals in the roots and reduce translocation to the shoot (Cornejo et al., 2013). This involves several mechanisms, such as differentiated effects on host plant growth and AMF production of glycoproteins, called glomalins, which have high metal retaining capacity (Melo et al., 2015).

Grain Cu content exhibited a positive quadratic response for all treatments; however, inoculations with mycorrhizal fungi were significantly lower than that of the control (Figure 4 C). Mycorrhizal fungi protect plants against metal toxicity (Soares & Siqueira, 2008), since they can compartmentalize copper in spores, reducing its translocation to other plant organs (Cornejo et al., 2013), and produce glomalin, which is efficient in retaining and reducing translocation of the contaminant (Melo et al., 2015). According to Decree no. 272.688, of August 27, 1965, the Cu tolerance limit in cereals (dry matter) is 30 mg kg^{-1} (Agência Nacional de Vigilância Sanitária [ANVISA], 1965), which is the same for grains (Associação Brasileira das Indústrias da Alimentação [ABIA], 1985). In all treatments, Cu levels in wheat grains were below the maximum permissible limit in Brazil, regardless of the dose applied to the soil.

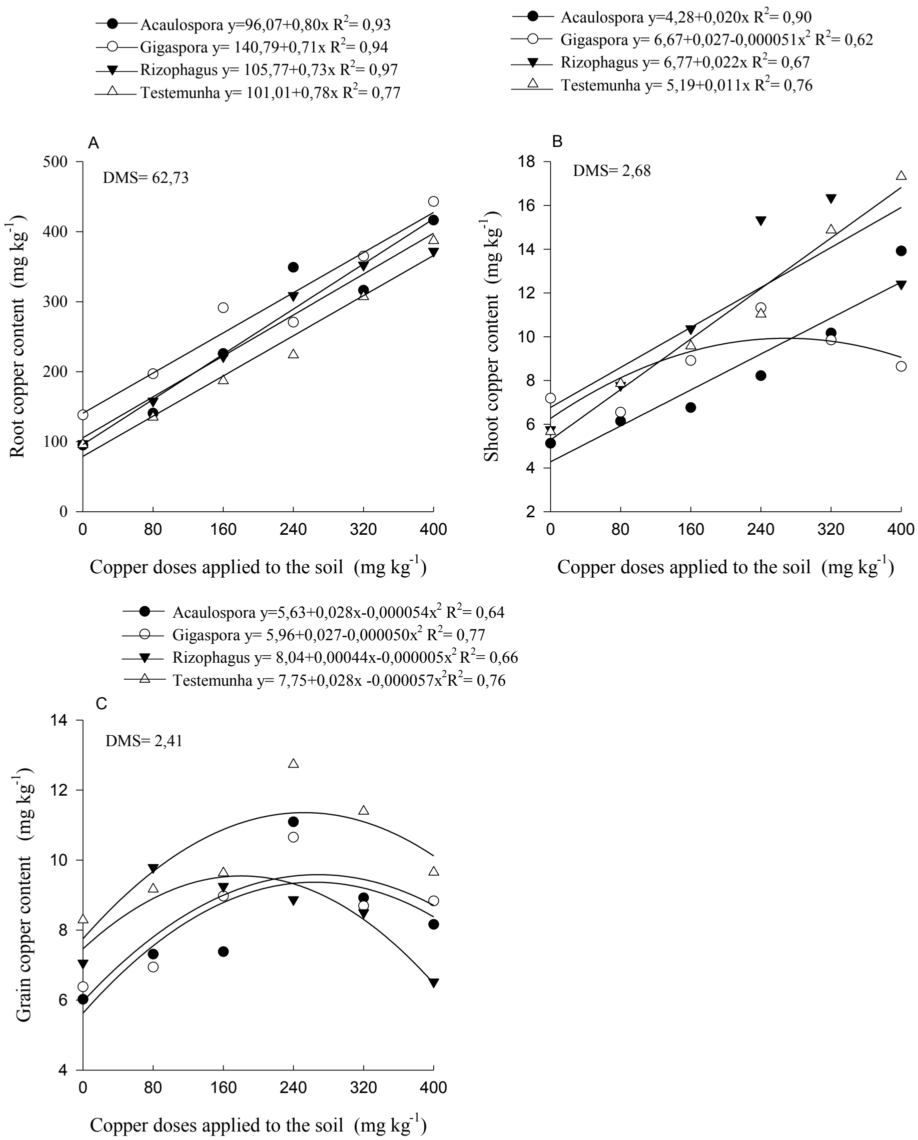

Figure 4. Root (A), shoot (B) and grain (C) copper content of wheat plants grown with different copper doses and inoculated with *Acaulospora Colombiana, Gigaspora Margarita* and *Rizophagus Clarus.*

The results showed a significant interaction between inoculum and doses for mycorrhizal colonization rate, with a linear reduction for the three isolates, being higher with *A. Colombiana,* but surpassed by *G. Margarita* at the highest dose tested (Figure 5). Evaluating ten different AMF isolates, Graham and Abbott (2000) found a wheat colonization rate of 50 and 89% for *Acaulospora* and *Gigaspora* isolates, respectively. In another study with *Acaulospora* and *Glomus* isolates, Sala et al. (2007) found colonization levels of 38 and 39%, respectively. The percentage of mycorrhizal colonization is related to the specificity and affinity of the fungus with the host, which may cause a difference in the effect of colonization (Matsubara et al., 2009).

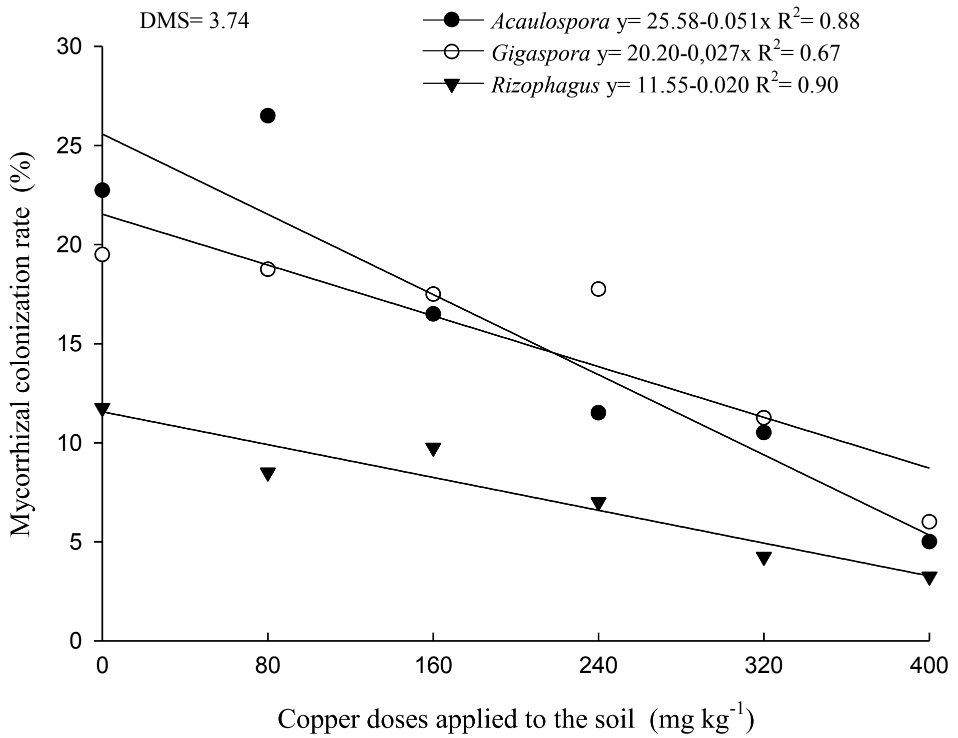

Figure 5. Percentage of mycorrhizal colonization of wheat roots inoculated with *Acaulospora Colombiana, Gigaspora Margarita* and *Rizophagus Clarus* cultivated with different doses of copper (0, 80, 160, 240, 320, 400 mg kg^{-1} of soil).

The decline in mycorrhizal colonization rates with inoculations of *A. Colombiana, G. Margarita* and *R. Clarus* was 78, 70 and 72% when compared to doses of 0 and 400 mg kg^{-1} of Cu, respectively. High heavy metal concentrations reduce the mycorrhizal colonization rate (Andrade & Silveira, 2004). In grapevines, the increase in soil copper concentrations reduced AMF colonization by 70% due to the toxic effect of this metal (Rosa et al., 2016). Even with reduced colonization, the effect of mycorrhization is evident and beneficial to plants in metal contaminated soil, resulting in greater plant height, root length, specific surface area and average root diameter in relation to non-mycorrhizal plants (Kumar et al., 2015; Smith & Read, 2008; Costa, & Melloni, 2019).

Conclusion

The mycorrhizal isolates *Acaulospora Colombiana* and *Gigaspora Margarita* provide greater wheat height, root length and specific surface area, when compared to Rizophagus Clarus and the control.

Mycorrhizal colonization with the fungi *Acaulospora Colombiana, Gigaspora Margarita* and *Rhizophagus clarus* resulted in lower wheat shoot and grain Cu content.

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