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Co-inoculation of *Azospirillum* with mycorrhizal fungi in the cultivation of wheat in soils contaminated with copper

Coinoculação de *Azospirillum* com fungos micorrízicos no cultivo de trigo em solo contaminado com cobre

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Highlights

The copper content in the grain increased significantly for all isolates when compared to doses of 0 and 400 mg kg^{-1} of copper in the soil.
Inoculation of wheat seeds with *A. brasilense* promotes an increase in tillering, plant height, and productivity compared to mycorrhizal fungi.
Co-inoculation of *A. brasilense* with mycorrhizal fungi, regardless of the species (A. colombiana and A. brasilense), does not contribute to the reduction of copper in the aerial part of wheat plants.

Abstract

Copper is a micronutrient essential for plant growth since it is part of the constitution of enzymes and proteins. However, it can become toxic to plants when in high concentrations in the soil. The association between microorganisms and plants is an alternative for reducing the negative effects of excess copper

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on plants. The objective of this work was to determine the influence of inoculation and co-inoculation of arbuscular mycorrhizal fungi and *Azospirillum brasilense* in a wheat crop grown on soil contaminated with copper. The experimental design used was entirely randomized in a bifactorial arrangement of 5 x 2, with five sources of inocula, (*Azospirillum brasilense; Acaulospora colombiana; Gigaspora margarita; Acaulospora colombiana + Azospirillum brasilense; Gigaspora margarita + Azospirillum brasilense*;), two doses of copper (0 = natural soil content and 400 mg kg^{-1}), and eight replicates. We evaluated the height of plants, number of tillers, root length, root volume, dry root mass, specific surface area, average root diameter, copper content in the root, straw and grain, productivity, and percentage of mycorrhizal colonization. The inoculation with *A. brasilense* increases the number of tillers, plant height, and productivity compared to mycorrhizal fungi. The co-inoculation of these microorganisms does not contribute positively to these evaluated parameters, regardless of the dose of Cu applied to the soil. Co-inoculation of *A. brasilense* with mycorrhizal fungi does not reduce copper in the aerial part of wheat plants, regardless of the evaluated species, even in soil contaminated with copper.
Key words: *Azospirillum brasilense.* Copper. Mycorrhizal fungi. Wheat.

Resumo

O cobre é um micronutriente essencial para o crescimento dos vegetais, pois faz parte da constituição de enzimas e proteínas, no entanto, quando em elevadas concentrações no solo pode se tornar tóxico às plantas. A associação entre microrganismos e plantas é uma alternativa para redução dos efeitos negativos do excesso de cobre às plantas. O objetivo deste trabalho foi avaliar a influência da inoculação de bactérias promotoras de crescimento vegetal e sua coinoculação com fungos micorrízicos arbusculares e na cultura do trigo cultivado em solos contaminados com cobre. O delineamento experimental utilizado foi inteiramente casualizado em arranjo bifatorial 5 x 2, sendo cinco fontes de inóculos (*Azospirillum brasilense; Acaulospora colombiana; Gigaspora margarita; Acaulospora colombiana + Azospirillum brasilense; Gigaspora margarita + Azospirillum brasilense*;); duas doses de cobre (0 = teor natural do solo e 400 mg kg^{-1}), com 8 repetições. Avaliou-se a altura de plantas, n° de perfilhos, comprimento de raiz, volume de raiz, massa seca de raiz, área superficial específica, diâmetro médio de raiz, teor de cobre na raiz, palha e grão, produtividade e porcentagem de colonização micorrízica. A inoculação com A. brasilense promove aumento do perfilhamento, altura das plantas e produtividade em comparação aos fungos micorrízicos e a coinoculação desses microrganismos não contribui positivamente para os parâmetros avaliados, independente da dose de cobre aplicada no solo. A coinoculação de A. brasilense com fungos micorrízicos, independente da espécie avaliada não contribui para a redução de cobre na parte aérea das plantas de trigo, mesmo em solo contaminado com cobre.
Palavras-chave: *Azospirillum brasilense.* Cobre. Fungos micorrízicos. Trigo.

Introduction

Copper (Cu) is a micronutrient essential to plants, acting on respiration, photosynthesis, and biological nitrogen fixation (Magalhães & Weber, 2021). Liquid pig manure has been used as a source of organic fertilizer in wheat crops as a low-cost alternative (Fernandes et al., 2017). However, successive applications lead to an accumulation of certain nutrients, among which is copper, which can cause chemical changes in the soil, toxicity, and nutritional imbalance in crops (Magalhães & Weber, 2021). Thus, No. 85/2014 established the reference value and soil quality of agricultural areas with the presence of volcanic rocks within the plateau of the state of Rio Grande do Sul at 203 mg kg^{-1} of total copper (Fundação Estadual de Meio Ambiente do Rio Grande do Sul [FEPAM], 2014), requiring alternatives to enable the cultivation of crops in areas contaminated with copper.

Wheat (*Triticum aestivum*) is the second most-produced cereal worldwide and the primary source of protein and calories in many countries (Wang et al., 2012). Brazil cultivated 2.2 million hectares per harvest between 2009 and 2019, with an average production of 5.4 million tons per year, cultivated mainly in the south, southeast, and midwest regions, where it is the most important winter crop, accounting for 87.3% of Brazilian production (Companhia Nacional de Abastecimento [CONAB], 2020). However, excess copper reduces plant development, decreasing production capacity due to damage caused to the photosynthetic apparatus, interfering with chlorophyll biosynthesis, cell division, and water and nutrients absorption and translocation capacity (Martins et al., 2014), also affecting tiller emission capacity, which is directly correlated with wheat production (Valério et al., 2009).

The inoculation of bacteria of the genus *Azospirillum* has been studied as an alternative to reduce costs with wheat fertilization due to the potential of fixing N_2 (Numoto et al., 2019; Medeiros et al., 2007). However, these bacteria can also promote hormonal changes and induce greater root growth and consequently greater absorption of water and nutrients (Bashan & Bashan, 2005), denominated plant growth promoter bacteria (Milléo & Cristófolli, 2016). Several studies demonstrate the ability of different genera of bacteria to grow in soils with high concentrations of metals (Uzel & Ozdemir, 2009; Chen et al., 2008; Florentino et al., 2009), thus being an alternative for wheat cultivation in soils contaminated with copper.

Arbuscular mycorrhizal fungi (AMF) are mandatory biotrophic organisms that establish a symbiotic relationship with a large number of plant species (about 80%), (Smith & Read, 2008), thus contributing to the development, production, and protection against pathogens, increased resistance to environmental stress, and a reduction in the use of fertilizers and pesticides in crops, as well as growth due to their ability to produce and accumulate bioactive compounds (Lins et al., 2007; Caproni et al., 2005). Additionally, their hyphae retain metals, protecting plants from harmful effects (Andreazza et al., 2013).

In this context, the doubt that persists is whether the inoculation of plant growth promoter bacteria (*Azospirillum brasilense*) and its co-inoculation with arbuscular mycorrhizal fungi (AMF) can promote the development of wheat in soils contaminated with copper. The objective of this work was to evaluate the influence of the inoculation of plant-growth-promoting bacteria and their co-inoculation with arbuscular mycorrhizal fungi on wheat grown in soils contaminated with copper.

Material and Methods

The experiment was conducted in the Department of Forestry Engineering greenhouse of the Universidade Federal de Santa Maria (UFSM), Frederico Westphalen Campus, located at the following geographical coordinates: latitude 27o 23′ 47″ S; longitude 53o 25′ 45″ W and altitude of 488 m, between March and November 2020. The soil used in the experiment was characterized as a Red Latosol, collected in an agricultural production area in the 0 – 20 cm layer, with a texture of 70% clay. However, medium sand was added at a 30% (v/v) ratio to facilitate root analysis, obtaining a texture of 52.5% clay. Subsequently, a sample of this substrate was taken to determine chemical and physical attributes (Table 1).

Table 1
Clay content and chemical analysis of the soil used for cultivating wheat

-- Parameters --								
Clay	pH	OM	P	K	Cu	Zn	Mg	Al+H
%	1:1	%	--------------- mg/dm³ ---------------			----- cmolc/L ------		
52.5	5.2	0.9	0.85	24.97	8.1	0.59	1.92	1.85

After the soil analyses, limestone was applied to correct the pH in water to 6.5, and fertilization was performed using a chemical fertilizer with formulation NPK 10-20-20 for productivity expectation of four tons, following the recommendations of the *Manual de Adubação e Calagem para o Rio Grande do Sul e Santa Catarina* Comissão de Química e Fertilidade do Solo [CQFS] (2016). Soil contamination with copper occurred 30 days after adding limestone, using copper sulfate homogenized by stirring in plastic bags, and incubated for 60 days for stabilization.

Arbuscular mycorrhizal fungi (AMF) were derived from the Glomeromycota International Culture Collection (GICC) of the Mycorrhizal Plants Laboratory of the Universidade Regional de Blumenau – SC. Inoculation was performed at the time of sowing the wheat, in which 30 spores were added to the seed per pot of each of the isolates.

Azo Total Max® liquid inoculant composed of bacteria of the genus *Azospirillum brasilense* (AbV5 e AbV6) was used in the concentration of 2,000,000 CFU, homogenized with the seed at the time of sowing.

The wheat cultivar used was TBIO Sinuelo, using 5-liter plastic pots filled with 5 kg of soil for the plant's cultivation in the greenhouse. Sowing was carried out in the second half of June, with ten seeds placed in each pot. Thinning was carried out 15 days after germination, leaving four plants per pot (experimental unit – EU) until the end of the productive cycle of the crop, considered the end of the experiment. Irrigation was controlled daily by an automatic drip system, keeping the soil at 80% of the field capacity.

The experimental design used was completely randomized in a bifactorial arrangement of 5 x 2, with five sources of inocula (*Azospirillum brasilense; Acaulospora colombiana; Gigaspora margarita; Acaulospora colombiana + Azospirillum brasilense; Gigaspora margarita + Azospirillum brasilense*); two doses of copper (0 = natural soil content and 400 mg kg^{-1}), and eight replicates.

At the end of the crop cycle, the plants were collected, separating the aerial part (straw + grains) from the roots by cutting in the hypocotyl region. The roots were separated from the soil by washing with water and using 0.5 and 1.0 mm mesh sieves. Subsequently, we determined the root length (RL) in cm, the specific surface area (SSA) of the roots – according to Tennant (1975) –, the mean root diameter (MRD) by employing the SAFIRA computational program (Jorge & Rodrigues, 2008), and root volume (RV) by using a measuring cylinder. The straw (SDM) and root (RDM) dry mass were quantified after drying in a greenhouse at 60 °C until reaching constant masses and later weighed using an analytical scale. Grain yield was quantified after determining humidity and humidity correction to 13%.

Subsequently, the straw dry mass, grains, and roots were ground in Willey type mill with a 10-mesh sieve to determine copper contents through nitro-perchloric extraction (3:1) and quantification in atomic absorption spectrophotometry, as described by Miyazawa et al. (2009).

The methodology used to determine the percentage of mycorrhizal colonization (PC) was based on the coloration of roots with Trypan Blue 0.05% to visualize the colonization of arbuscular mycorrhizal fungi (AMF) in a stereomicroscope with a magnification of 60x (Brundrett, 2009). Colonization was estimated at five replicates per plant, totaling 35 replicates per treatment using the checkerboard method (Giovannetti & Mosse, 1980).

The results were submitted to analysis of variance and, when significant, the means were compared by the Scott Knott's test. The results were analyzed using the SISVAR statistical program (Ferreira, 2011), considering as reference the significance levels superior to 95%.

Results and Discussion

The variables plant height, number of tillers, specific surface area and mean root diameter did not show statistical difference between the variation factors for the Scott Knott test at a 5% probability of error (Table 2). The means of simple effects indicated that the addition of 400 mg of copper kg^{-1} in the soil significantly reduced the height, number of tillers, average diameter, and specific surface area of the roots compared to the zero dose (Table 2).

Toxic effects on plants by high levels of copper in the soil are reported in the literature (Santos et al., 2010; Freitas et al., 2020) and indicate a reduction in the ability to issue tillers, decreasing the productive capacity (Panziera et al., 2018). Although copper is an essential element for plant growth, it becomes toxic when in high concentrations in the soil, reducing plant productivity (Michaud et al., 2008).

Table 3

Average plant height (PH), number of tillers (NT), specific surface area (SSA), and average root diameter (AD) of wheat plants, grown in soils with two doses (0 and 400 mg kg^{-1} copper in soil), and sources of inoculum (*Azospirillum brasilense; Acaulospora colombiana, Acalospora Colombiana + Azospirillum brasilense; Gigaspora margarita* and *Gigaspora margarita + Azospirillum brasilense*)

Doses	PH (cm)	NT	SSA (mm^2)	AD (mm)
0	50.63 A	10.06 A	44,174.7 A	1.26 A
400	43.33 B	8.80 B	31,939.2 B	0.57 B
Inoculum				
Azospirillum b.	53.91 A	11.58 A	42,287.7 A	0.93 A
Acaulospora	45.66 C	9.50 B	39,811.1 A	0.86 B
Acaulospora+Azosp.	43.66 C	8.66 B	34,819.2 B	0.94 A
Gigaspora	48.33 B	9.16 B	36,143.8 B	0.81 B
Gigaspora+Azosp.	43.33 C	8.25 B	35,143.2 B	0.97 A
CV (%)	6.93	17.53	20.43	16.11

*Means followed by the same letter in the column do not differ from each other according to the Scott Knott test at 5% error probability.

In conditions of high exposure to copper, the activity of root meristems tends to decrease in plants with low tolerance to heavy metals (Pereira et al., 2013). Thus, there is a reduction in the transport capacity of photoassimilates to the roots, affecting their development and the plants' absorption capacity of water and nutrients (Castro et al., 2009).

The inoculation with *A. brasilense* allowed higher means for plant height (PH) and the number of tillers (NT) when compared to the addition of AMF in isolation or with co-inoculation between the two groups of microorganisms (fungi and bacteria). When inoculated with *Azospirillum brasilense,* increased tiller emission of wheat is considered a mechanism of yield promotion in response to inoculation (Fukami et al., 2016). Many studies have demonstrated the benefits of using inoculants of the genus *Azospirillum* in grasses, such as an increase in grain productivity, dry matter, plant height, straw diameter, tiller emission, and forage biomass (Andrade et al., 2016; Coelho et al., 2019; Hungria et al., 2016).

The specific root surface area was significantly larger with the isolated inoculation of A. brasilense and A. colombiana (Table 2). This effect may be related to competition between the AMF and bacteria during root colonization, given that when fungi and bacteria are inoculated together in cereals, both endophytes may be present in the same cortical area of the roots and enable a direct interaction between plant-bacterium-fungus, which may result in competition for photosynthetic compounds (Biró et al., 2000).

Inoculation with bacteria *A. brasilense* and co-inoculations *Acaulospora + Azospirillun* and *Gigaspora + Azospirullun* provided a greater average diameter of wheat roots (Table 2). The benefits provided to the root system of vegetables by inoculating AMF and diazotrophic bacteria include the increase in root system density, amount of root hairs, formation and development of roots, and changes in root architecture (Lima et al., 2015; Morais et al., 2015; Souza et al., 2017).

There was a statistical difference for the 5% Skoot Knot test between copper doses and sources of inocula for root volume (RV), root length (RL), and root dry mass (RDM) of wheat, with all these variables reduced with the addition of copper to the soil (Table 3). The reduction in the growth and development of the root system is one of the most obvious symptoms of the toxic effect of copper on plants.

Table 3

Root volume (RV), root length (RL), and root dry mass (RDM) of wheat plants with two doses (0 and 400 mg kg^{-1} copper in soil), and sources of inocula (*Azospirillum brasilense; acaulospora colombiana, acaulospora colombiana + Azospirillum brasilense; gigaspora margarita* and *Gigaspora margarita + Azospirillum brasilense*)

Inoculum	RV (cm³)		RL (cm)		RDM (g)	
	0	400	0	400	0	400
Azospirillum b.	24.66 bA	17.83 aB	39.00 aA	25.16 cB	10.63 aA	7.39 aB
A. colombiana	23.66 bA	20.16 aB	38.50 aA	31.50 aB	9.65 aA	6.27 bB
Acau+Azosp	18.00 cA	16.83 aA	37.00 aA	25.50 cB	7.53 bA	5.79 bB
G. margarita	25.50 aA	17.00 aB	38.16 aA	31.00 aB	9.71 aA	8.40 aB
Gig+Azosp	22.16 bA	17.00 aB	36.83 aA	28.50 bB	8.21 bA	4.94 bB
CV (%)	13.48		6.79		13.29	

*Means followed by the same lowercase letter in the column and uppercase letter in the line do not differ from each other according to the Scott Knott test at 5% error probability.

However, at the zero dose of the metal, inoculation with *G. margarita* allowed a greater root volume of the wheat (Table 3). The mycelium-connected network of the AMF can increase root volume, thus increasing the area of exploited soil and plant absorption of water and nutrients (Smith & Read, 2008).

The isolated inoculation of bacterium *A. brasilense* and AMF *G. margarita* and *A. colombiana,* at zero dose, and *A. brasilense* and *G. margarita,* with 400 mg kg^{-1} of copper in the soil, induced greater wheat root dry mass (Table 3). Mycorrhizal fungi establish a relationship between the root of the host plant and the mycelium (Brundrett, 2009), increasing the length, volume, and dry mass of roots, allowing greater absorption of water and nutrients by the plants (Zhao et al., 2015; Aguegue et al., 2017; Silva et al., 2018). The effect of AMF on root length was also evidenced in the present work.

Azospirillum, in turn, colonizes the rhizosphere and internal tissues and produces phytohormones and substances that lead to the protection and increase of the root system (Cohen et al., 2015). Effects on the morphology of roots colonized by diazotrophic bacteria are reported in the literature, such as the increase in root length, number of branches, root hairs, and specific surface area of the roots (Roesch et al., 2005).

The results showed a significant interaction between copper doses and sources of inocula for wheat productivity, being greater with the inoculation of *A. brasilense* at zero dose of copper in the soil (Figure 1A). *A. brasillense* performs the function of biologically fixing nitrogen and increasing the absorption surface of plant roots and volume of the exploited soil (Repke et al., 2013; Milléo & Cristófolli, 2016). Tiller emission of wheat plants is also higher when inoculated with *A. brasilense,* as demonstrated in the present work (Table 2), and this increase is considered a mechanism of promotion in yield in response to inoculation (Fukami et al., 2016).

At a dose of 400 mg kg^{-1} of copper, the sources of inocula did not differ in grain productivity. However, productivity significantly reduced by around 30% when compared to the zero dose (Figure 1A). The emission of tillers is directly correlated with the production of wheat plants (Valério et al., 2009), and soil contamination with copper reduced tiller emission (Table 2). When in high concentrations in the soil, copper affects several morphophysiological processes in plants, including the reduction of photosynthetic rate, cell division, and the capacity of absorption and translocation of water and nutrients (Martins et al., 2014). These results demonstrate that copper becomes toxic when in high concentrations, reducing the productive capacity of plants (Michaud et al., 2008).

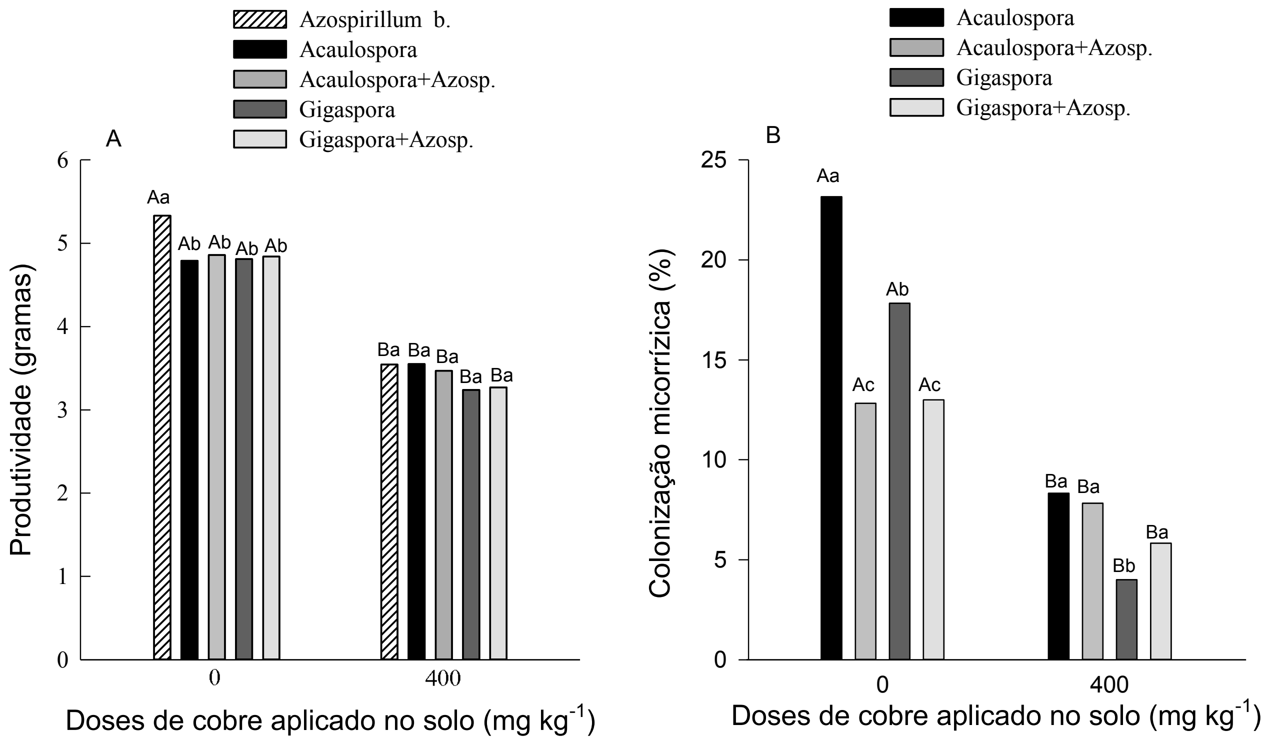

Figure 1. Grain productivity (A) and percentage of root mycorrhizal colonization (B) of wheat grown under copper doses (zero, natural content, and 400 mg kg^{-1} soil) and sources of inocula (*Azospirillum brasilense; Acaulospora colombiana, Acaulospora colombiana + Azospirillum brasilense; Gigaspora margarita,* and *Gigaspora margarita + Azospirillum brasilense*).
*Means followed by the same uppercase letter between doses and lowercase letter in the same dose do not differ from each other according to the Scott Knott test at 5% error probability.

The results showed a statistical difference between the sources of inocula and the doses of copper for the percentage of mycorrhizal colonization, being reduced with the dose of copper (Figure 1B). In the treatment with no copper application, the percentage of mycorrhizal colonization was higher in the treatments containing the fungi inoculated in isolation compared to the co-inoculations, highlighting *A. colombiana,* which presented 24% of colonized roots (Figure 1B). The colonization rate of wheat roots is higher when only AMFs are inoculated. This reduction in colonization in co-inoculation treatments occurs due to competition between microorganisms (Sala et al., 2007). *Azospirillum* spp. and the AMF can be present in the same cortical area of the roots, making possible direct interaction between plant-bacterium-fungus, resulting in competition for photosynthetic compounds (Biró et al., 2000).

Fungus *A. colombiana* and the co-inoculations of AMF and bacteria showed greater mycorrhizal colonization in the dose of 400 mg of copper kg^{-1} in the soil (Figure 1B). Mycorrhizal colonization is directly affected by high doses of copper in the soil, which negatively affects its metabolic processes (Banu et al., 2004). High doses of copper in the soil also reduced mycorrhizal colonization in *Eucalyptus grandis* seedlings (Antoniolli et al., 2010) and vine roots (Rosa, 2019).

The copper content in the root (CuR), straw (CuS), and grain (CuG) showed a statistical difference between the isolates and the copper doses applied in the soil (Table 4). The copper content in the wheat root increased with the application of the metal in the soil and indicated that the inoculation of *A. brasilense, A. colombiana,* and *G. margarita* presented an increase three- to four-times greater in the accumulation of this metal in the root. The use of heavy metal-resistant rhizobacteria is an alternative that can assist in the development and growth of plants in contaminated soil and is also an important strategy to recover areas contaminated with copper (Rajkumar & Freitas, 2008; Estrela et al., 2018).

Table 4
Copper content in the root, straw, and grain of wheat plants with two doses (0 and 400 mg kg^{-1} copper in the soil), and sources of inocula *(Azospirillum brasilense; acaulospora colombiana, acaulospora colombiana + Azospirillum brasilense; gigaspora margarita, and Gigaspora margarita + Azospirillum brasilense)*

Inoculum	CuR (mg kg^{-1})		CuS (mg kg^{-1})		CuG (mg kg^{-1})	
	0	400	0	400	0	400
Azospirillum b.	119.5 bB	440.4 aA	5.61 aB	13.84 bA	8.26 aB	10.91 aA
A. colombiana	92.5 bB	419.9 aA	5.28 aB	8.68 cA	5.97 bB	8.35 bA
Acau+Azosp	156.8 aB	349.5 bA	4.96 aB	17.26 aA	8.14 aA	8.93 bA
G. margarita	143.1 aB	451.7 aA	7.51 aA	8.77 cA	6.43 bB	8.77 bA
Gig+Azosp	124.9 bB	333.1 bA	7.13 aA	9.53 cA	8.62 aB	11.71 aA
CV (%)	12.85		25.31		16.47	

*Means followed by the same lowercase letter in the column and uppercase letter in the line do not differ from each other according to the Scott Knott test at 5% error probability.

Arbuscular mycorrhizal fungi play an important role in protecting against plant toxicity by heavy metals since they have mechanisms that prevent toxicity, such as copper compartmentalization in spores, consequently reducing the availability of Cu in the roots (Soares & Siqueira, 2008; Cornejo et al., 2013). Another mechanism is the production of glomalin – a glycoprotein that is secreted and causes metal complexation (Folli-Pereira et al., 2012). The effects are also mitigated with the improvement of the nutritional status of the plants and the formation of metal-phosphate compounds, complexing the metals in the roots and avoiding their transport to the aerial part (Soares & Siqueira, 2008).

The copper content in the straw was significantly higher with the addition of 400 mg kg^{-1} of the metal to the soil, emphasizing co-inoculation with *A. colombiana* + *A. brasilense,* followed by *A. brasilense* with 17.26 and 13.84 mg kg^{-1} of copper, respectively (Table 4). Diazotrophic bacteria and mycorrhizal fungi can develop different resistance mechanisms to copper. Therefore, the association of MAF and diazotrophic bacteria favors the growth of plants in contaminated environments and can be used as an alternative to removing copper from the soil (Andreazza et al., 2010, 2013). Copper deficiency in such a crop can cause male sterility of flowers when the copper content in the leaf is less than 3.3 mg kg^{-1}, with normal concentrations ranging from 5 to 20 mg kg^{-1} (Bona et al., 2016). In this sense, the cultivation of wheat inoculated with fungi and bacteria in soils contaminated with 400 mg of copper kg^{-1} in the soil did not exceed the levels considered normal.

The copper content in the grain increased significantly for all isolates compared to doses of 0 and 400 mg kg^{-1} of copper in the soil, being superior to the inoculation of *A. brasilense* and the fungus/bacterium co-inoculations (Table 4). Heavy metal-resistant rhizobacteria can assist in acquiring contaminants by improving plant growth (Estrela et al., 2018). On the other hand, mycorrhizal fungi allowed a lower copper content in the grain at the dose of 400 mg kg^{-1} of copper in the soil.

Mycorrhizal fungi protect metal toxicity by compartmentalizing copper in spores and producing glomalin, a substance that reduces the translocation of the contaminant (Cornejo et al., 2013). Another characteristic that reduces the mobilization of the element to the grain is the phytostabilization and immobilization of metals at the root (Soares & Siqueira, 2008; Santos et al., 2010). According to Decree No. 55,871 of March 26th, 1965, (Agência Nacional de Vigilância Sanitária [ANVISA], 1965) and the Associação Brasileira das Indústrias de Alimentação [ABIA] (1965), the maximum tolerance limit (LMT) for copper in food is 30 mg kg^{-1}. Therefore, the levels of copper in the wheat grain were below the maximum limits provided for by the legislation for all the treatments.

Conclusion

Inoculation with *A. brasilense* promotes an increase in the number of tillers, plant height, and productivity compared to mycorrhizal fungi, and the co-inoculation of these microorganisms does not contribute positively to these evaluated parameters, regardless of the dose of Cu applied to the soil.

Co-inoculation of *A. brasilense* with mycorrhizal fungi does not reduce copper in the aerial part of wheat plants, regardless of the species (*A. colombiana* and *Gigaspora margarita*), even in soil contaminated with copper.

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