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Mineral nutrition of mycorrhized seedlings of a tropical gum tree in copper contaminated soil

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ABSTRACT

Extensive use of such fungicides leads to Cu diffusion in most soils and increases risk of Cu phytotoxicity for the tropical gum tree (*Sterculia setigera*) grown in these soils. A pot experiment was set to examine the effect of Arbuscular Mycorrhizal Fungus (AMF), *Rhizophagus fasciculatus*, and soil copper levels content on mineral status of the tropical gum tree *Sterculia setigera*. Plants were grown in soil with different Cu levels (0, 200, 400, 600 and 800 mg·kg^{-1}). Control plants (C) were found to have survival rate were of 40% at 600 mg.kg^{-1} Cu. Inoculated plants (T) on their part had a survival rate of 100% on Cu contaminated soils up to 600 mg·kg^{-1} Cu. However, at 800 mg.kg^{-1} Cu level, 100% of the plants died. Root colonization rates (7.4%) were significantly lower at 600 mg.kg^{-1} Cu. In contaminated soil, higher mycorrhizal colonization was measured at 0, 200, and 400 mg·kg^{-1}Cu addition levels. AMF-inoculated plants had higher K, P, N, Ca, Mg and Cu concentrations than uninoculated plants. In mycorrhized plants, nutrient concentrations increased with the increasing levels of soil Cu and were higher than those of the non-mycorrhized plants. Unlike Na, the uptake of K increased in shoot tissues of mycorrhizal plants with the increasing levels of Cu. Results suggested that *S. setigera* associated with AM fungus *Rhizophagus fasciculatus* had the potential to survive and to grow under moderately Cu contaminated soil system.

Keywords: Arbuscular mycorrhiza, *Sterculia setigera*, Copper, Soil, Heavy Metal.

INTRODUCTION

Excessive concentrations of heavy metals in the environment are known to be toxic to most organisms and their effects on organisms are being increasingly studied (Malekzadeh et al., 2012; Kanwal et al., 2016). Effects of heavy metal contamination on plants result in growth inhibition, structural damage, and a decline of physiological and biochemical activities. Copper (Cu) is an essential micronutrient for plants (He et al., 2016, Marastoni et al., 2019). However, high

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contents of this element can be toxic not just to plants and microorganisms, but also to humans through the food chain and pose a potential threat to human health, environmental quality, and sustainable food production (Tao et al., 2003; Menti et al., 2006 Adrees et al., 2015; Kumar et al., 2021; Rillig et al., 2023). Intensive and repeated use of copper in agriculture as fungicides is the main source of Cu contamination (Fuente et al., 2021, Brunetto et al., 2019). Extensive use of such fungicides leads to Cu diffusion in most soils and increases risk of Cu phytotoxicity for the tropical gum trees as *Sterculia setigera* grown in these soils. Song et al. (2004) have shown that plants grown on Cu contaminated soil can develop mechanism to resist or tolerate Cu inside their cells. *Sterculia setigera* is a tropical gum plant belonging to family *Sterculiaceae* with a wide natural distibution in Sudano-Guinean zone of Africa (Johnson et al., 2005). This plant species has socio-economic and cultural importance in sub-Saharan Africa (Johnson et al., 2005; Betty et al., 2011). Its exudate karaya gum is mostly extracted from private parkland and forests in Senegal which is the world second largest exporter after India (Benjamin and Wilshusen, 2007). Within these countries, gum exploitation is a valuable source of income for many indigent smallholders (Touré et al., 2009). *Sterculia setigera* is a high biomass tree able to develop Arbuscular mycorrhizal fungi (AMF) symbiosis (Ndiaye et al., 2011). AMF are ubiquitous in both natural and agricultural ecosystems, including in sites contaminated with heavy metals (Quilambo, 2003). They provide a direct link between soil and roots, and therefore have a crucial contribution to plant growth by improving mineral nutrition and plant tolerance to stress (Gaur and Adholeya, 2004; Ferreira et al., 2015; Hodge and Storer, 2015;). AMF also affect the uptake of metals and transfer of the root (Hassan et al., 2013, Riaz *et* al., 2021). Mycorrhizal fungi can also have an important role in mitigating metal toxicity in plants (Ambrosini et al., 2015; Jin et al., 2015).

It is in Highlight that the aim of the present study was to evaluate whether AMF can improve the mineral nutrition of tropical gum tree under Cu contaminated soil.

MATERIALS AND METHODS

Physico-chemical characteristics of soil

The soil used for pot culture in this study was collected at 5-20 cm depth from the Botanical Garden of Plant Biology Department situated at latitude 14°41’2’’N, longitude 17°27’45’’W (University Cheikh Anta Diop/Senegal). Soil characteristics are given in Table 1.

Mycorrhizal inoculum

AMF inoculum containing indigenous species *Rhizophagus fasciculatus* was obtained from the Laboratory of Fungal Biotechnology (LBC) of the department of Plant Biology (Universty Cheikh Anta Diop / Senegal). AMF was multiplied by using maize as host plant. AMF inoculum consisted of rhizospheric soil mixture from culture containing spores, hyphae and mycorrhizal root fragments (an average of 40 spores per gram and 85% of roots infected).

Experimental procedure

Soils were first sterilized by autoclave at 120°C for 1h. Experiment treatments consisted to thoroughly mixing soil and Cu SO_4 (analytical grade) at 5 different levels: 0, 200, 400, 600 and 800 $mg.kg^{-1}$. Each nursery bag containing 2 kg sandy soil of garden of the Botany (Table 1). Experiments were done in a randomized block with five replicates. Two factors were studied: (a) Cu addition level and (b) inoculation. Seeds of *S. setigera* were scarified by the addition of sulfuric acid (H_2SO_4 96%) for 100 min, and rinsed in sterile distilled water. After successive 5 min baths in sterile distilled water, seeds were germinated in jars. The jars were previously sterilized by autoclaving at 120°C for 20 min and contained soaked cotton. Germination occured in the dark at 32°C for 3 days. Two seedlings of *S. setigera* were then transferred in Cu contaminated nursery bags and only one seedling was left after emergence. During this procedure, plants were inoculated with AM fungus *R. fasciculatus* by placing 20 g of inoculum directly in the substrate at the position of the roots (the control without AM fungal propagules). Plants were grown in greenhouse with the following conditions: day/night cycle of 12/12h, 32/25°C and 40-50% air humidity. Plants received tap water.

Plant and soil analyses

Twelve weeks after sowing, plant shoots and roots were harvested separately. Sub-samples of fresh roots were analysed to assess root colonization rate. Shoots and roots were first rinsed with tap water and then with distilled water. Mycorrhizal colonization was evaluated using grid-line intersect method (Giovannetti and Mosse, 1980) after clearing with 10% KOH (Phillips and Hayman, 1970).

Chemical analyses of shoot and soil were conducted at Qualio, a certified laboratory in chemical analysis at the University of Franche-Comte in France. Cu, Ca, Na, Mg, and K concentrations in dried and ground plant material were determined by Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) after wet-digestion with a mixture of concentrated HNO_3 and $HClO_4$ (3:2, v/v, analytical grade) mixed acid. Kjeldahl nitrogen was determined by volumetry according to the standardized method NF EN 25663, ISO 5663: 1994. Phosphorus was determined by atomic absorption (GANIMEDE P from Hatch) using a molecular adaptation of the standardized method ISO 6878: 2005, and pH was determined in a 1:2.5 (w/v) soil/water suspension.

Statistical analyses

Statistical procedures were carried out with the software package R version 2.5. Two factors analysis of variance (ANOVA) was performed to partition the variance into the main effects and the interaction between inoculation and Cu level.

Table 1: Characteristics of the soil used in this study.

Components	**Contents**
Clay (%)	3.6
Silt (%)	1.6
Fine silt (%)	2.9
Fine sand (%)	51
Coarse sand (%)	40.9
Organic matter (%)	1.06
Total carbon (%)	2.5
Total nitrogen (%)	0.33
Conductivity (µS/cm)	658
Total phosphorus (ppm)	47
Available phosphorus (ppm)	3.1
pH (sol/water ratio 1:2)	6.7
pH (sol/KCl ratio 1:2)	4.5

RESULTS

Root colonization and survival rate

Root colonization rate did not differ for AMF inoculated plants that received 0 to 400 $mg.kg^{-1}$ of Cu. However, it strongly decreased with 600 $mg{\cdot}kg^{-1}$ Cu (Table 2). Control plants (C) were found to present survival rate at 40% at 600 $mg.kg^{-1}$ Cu. While, inoculated plants (T) were found at a survival rate of 100% on Cu contaminated soils up to 600 $mg{\cdot}kg^{-1}$ Cu. However, at 800 $mg.kg^{-1}$ Cu level, all plants died whatever the treatment.

Mineral nutrient uptake

Nutrient concentrations in the shoots of the seedlings after harvesting are shown in Table 3. *S. setigera* shoot mineral contents showed that the nutrient uptake nutrition differed according to the treatment. Colonization by *Rhizophagus fasciculatus* improved the content of these nutrients more than the control. The greatest differences among inoculated plants were found for foliar Ca Mg, N and P. In contrast inoculation reduced the contents of Cu and Na. No significant difference in Ca, K, Mg, Cu, N was observed at 0 and 600 mg/kg Cu levels. However, a decrease in Ca, Mg, Cu, N and P was observed at 800 mg/kg Cu (Table 3). Shoot K, and Cu concentrations for Cu level conditions were significantly higher than 800 mg/kg. Analysis of variance was seen to have significantly increased Ca, K, Mg, N and P foliar nutrient concentration by inoculation and Cu level status. Statistical results also show that combined factors were significant for the Ca, Mg, N and P contents. Ca, Mg concentration declined with increasing Cu level conditions (Table 3).

Table 2: Root colonization ratio (means ± SD) and survival rate of *Sterculia setigera* plants at different Cu addition levels. Non inoculated (C) and inoculated (T) with mycorrhizal fungus treatment.

Cu addition level ($mg{\cdot}kg^{-1}$)	Treatment	Root colonization rate (%)	Survival rate (%)
0	C	0	100
	T	65.40 ±2.6	100
200	C	0	100
	T	62.7 ±2.8	100
400	C	0	100
	T	58.6 ±3.1	100
600	C	0	40
	T	7.4± 0.7	100
800	C	0	0
	T	0	0

Table 3: P-values of the two-way ANOVA test for the effects of the factors “Cu level” and “Mycorrhizal inoculation treatment” and their interaction of leaf mineral nutrition in *Sterculia setigera* plants.

	Leaf mineral nutrition (mg/kg dry matter)						
	Ca	K	Mg	Cu	Na	N	P
Inoculation							
Control	27192 [b]	5736 [a]	6070 [b]	488 [a]	4701 [b]	18 [b]	363 [b]
AMF	38212 [a]	7951 [a]	9907 [a]	75 [a]	2386 [a]	36 [a]	605 [a]
Cu level (mg)							
0	38286 [a]	5375 [b]	6851 [ab]	12 [b]	3443 [ab]	33 [a]	717 [a]
200	36881 [a]	5130 [b]	7416 [ab]	100 [b]	2249 [b]	26 [a]	660 [a]
400	37412 [a]	6592 [b]	10635 [a]	94 [b]	6156 [a]	33 [a]	586 [a]
600	27868 [ab]	6617 [b]	8015 [ab]	49 [b]	2426 [b]	31 [a]	261 [b]
800	23066 [b]	10503 [a]	4146 [b]	1154 [a]	2182 [b]	8 [b]	195 [b]
p-value :							
Ino	1.49e-08 ***	0.05826.	3.80e-10 ***	0.0745.	0.00318 **	2.06e-08 ***	1.26e-11 ***
Cu level	1.11e-08 ***	0.00752 **	0.396	0.0101 *	0.59067	3.53e-07 ***	2e-16 ***
Ino x Cu level	2.48e-09 ***	0.49916	8.01e-09 ***	0.0147 *	0.04537 *	4.64e-09 ***	1.18e-11 ***

Different letters indicate significant differences according to Tukey test. Significant effect: p- value ≤ 0.05

DISCUSSION

Many agricultural soils are contaminated by heavy metals and such situations need careful attention. Mycorrhizal symbiosis is a key component in helping plants to cope with adverse environmental conditions (Diagne et al., 2020). The increased in mineral nutrition of seedlings associated with *Rhizophagus fasciculatus*, as compared to non-inoculated ones confirms the mycorrhizal dependency of *Sterculia*. The high degree of mycotrophy of this plant was already shown by Ndiaye et al. (2011). Our results show a threshold effect of AMF on plants at copper addition levels between 400 and 600 mg·kg^{-1} Cu (Table 2). The presence of copper did not have a negative affect on AMF colonization. Even in the treatments with highly contaminated soils (600 mg.kg^{-1} Cu), AMF were able to colonize *Sterculia* plants (7%). Depressive effect of heavy metal on AMF colonization was already observed in tree seedlings transplanted to contaminated soil (Colpaert et al., 2004; Zhang et al., 2012; Ferrol et al., 2016). In agreement with these ideas, Lins et al. (2006), Ruscitti et al. (2017), and Betancur-Agudelo et al. (2023) showed that high levels of Cu prevent germination of spores and mycorrhizal colonization. Furthermore, according to some study, plants which form mycorrhizal associations, could improve more vigorously, even in contaminated soils (Gonzalez-Chavez et al., 2004; Al-Garny, 2006; Zhang et al., 2012). In fact, (Wu et al., 2007) found that AMF colonization was low to moderate (4.2-12.8%). Nevertheless, another field survey reported that AMF colonization rates between 40 and 80 % (Leung et al., 2007; Su´arez et al., 2023). Also, some authors have shown that no correlation between root colonization and level metal pollution (Audet and Charest, 2006; Cicatelli et al., 2010). Present results show that high Cu level reduced mycorrhizal colonization rate of *S. Setigera* seedlings (Table 2). Reasons for such a discrepancy are not known yet but could reflect the role of different culture conditions (duration of the experiment, plant density, pot volume) and AMF species. Indeed, agricultural soils are often overfertilised, especially in the case of soils with high levels of heavy metals where farmers increase pH which reduces metal bioavailability. However, origin and stress adaptation of fungi may have played a key role on root colonization and plant growth by fungi. Mycorrhizal plants may release more root exudates containing soil enzymes than that of non-mycorrhizal plants because of the larger root system and/or improved nutrition and/or resistances to stress of mycorrhizal plants (Liu et al., 2023). Moreover, the ability of AMF species to mitigate the stress caused by high soil Cu contents in plants have been attributed to a promotion on the absorption of water and nutrients, particularly P, through the roots (Soares and Siqueira, 2008; Andrade et al., 2010). The root surface area is mostly associated with nutrient absorption, and enhancement in root volume leads to better capability in nutrient absorption and eventually has positive effect on the plant growth (Bochicchio et al., 2015).

In fact, many research reported AM fungal inoculation resulted in enhanced plant growth, total uptake of N, P and many other nutrients (Christie et al., 2004; Wang et al., 2005; Hodge and Storer, 2015). However, the opposite effect was observed in the Cu-contaminated soil (Nogales et al., 2019). The toxic effect of Cu is more important in control plants. Cu critical levels found in previous works were fewer and largely variable as stressed (Kopittke and Menzies, 2006 ; Michaud et al., 2007; Santana et al., 2015). Inoculation of *S. setigera* seedlings with AMF *Rhizophagus fasciculatus* significantly increased their mineral nutrition compared to control plants. This result suggested that AMF, *R. fasciculatus* exerts a protective effect to Cu contamination and stimulated the mineral nutrition of *S. setigera* plants even 400 mg.kg^{-1} Cu level. Ruscitti et al. (2017) found that AMF inoculation increased the growth of plants cultivated in soil with high Cu contents due to the reduced translocation of the metal to the shoot. However, Xiao et al. (2016), inoculated *Glomus intraradices* into a soil with high levels of Cu and found that the AMF increased the total Cu concentrations in the shoots. This finding confirms that the effect of mycorrhizal fungi on Cu phytoremediation is dependent on the plant species, fungal species, soil characteristics as previously described by Santana et al. (2015).

The effect of AMF inoculation on plant nutrition under soil Cu concentrations was different from the one observed under non-contaminated soil conditions. This agrees with Meier et al. (2015), who found that the effect of different mycorrhizal treatments on the levels of shoot nutrients depended on soil Cu concentration. *S. setgigera* shoot K, Ca, Mg, Cu, N, Na and P contents showed that the nutrient uptake was a function of the applied treatment. Colonization by *R. fasciculatus* with the addition 200, 400 and 600 mgKg^{-1} Cu improved the content of these nutrients more than the non-inoculated plants. The same results were found by Ruscitti et al. (2017) for inoculated plants. However, the opposite effect was observed in the Cu-contaminated soil for N et P (Nogales et al., 2019). Analyses of variance (Table 4) showed that inoculation and Cu addition level had significant individual effect on Ca, N and P. Nevertheless, interaction between inoculation and Cu levels was also significant for Ca, Mg, Cu, N and P ($p<0.05$), suggesting that interaction between the two factors should be given importance as it could have based individual effects. Statistical analysis also showed that the interaction affected Ca, Mg, N, P more than Cu and Na.

The support of symbiotic associations between these fungi and tropical gum tree may be suggested in application of inoculation for successfully improve mineral nutrient uptake of seedlings in Sahelian zone agrosystem.

Conclusion

In conclusion, inoculation treatment showed a protective effect on host plants against copper. AMF also enhances foliar essential nutrient uptake under Cu contaminated soil. Finally, it may be concluded that *S. setigera* with AM fungus *Rhizophagus fasciculatus* have potential to survive and to grow under Cu moderately contaminated soil system.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

MN contributed on inception of the paper, research, and writing. EC contributed on inception and reviews of the paper. AGBM contributed on inception and reviews. TAD reviewed the work. All authors contributed to the article and approved the submitted version.

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