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Mycorrhization improves the mineral nutrition of *Sterculia setigera* plants growing on Zinc-contaminated soil

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ABSTRACT

Objective: This greenhouse study aimed to examine the effect of arbuscular mycorrhizal fungus (AMF), *Rhizophagus fasciculatus*, and soil zinc levels content on the mineral status of tropical gum tree *Sterculia setigera*.
Methodology and results: Plants were grown in soil under different Zn levels (0, 200, 400, 600 and 800 mg·kg^{-1}). They were harvested after three months of cultivation, and growth, root symbiosis, and mineral nutrient concentrations were evaluated. Control plants (C) have not been colonized their survival rate was found to be 45% at 600 mg.kg^{-1} Zn. Inoculated plants (T) were found to have a survival rate of 100% on Zn-contaminated soils up to 600 mg·kg^{-1} Zn. However, at 800 mg.kg^{-1} Zn levels, 100% of the plants died. Root colonization rates (8.5%) were significantly lower at 600 mg/kg Zn. Higher mycorrhizal colonization was measured in contaminated soil at 0, 200, and 400 mg·kg^{-1} Cu addition levels in AMF-inoculated plants. AMF-inoculated plants had higher K, P, N, Ca, Mg, and Zn concentrations than control plants. In mycorrhized plants, nutrient concentrations increased with the increasing levels of Zn soil and were higher than those of the non-mycorrhized plants. Unlike Na, the uptake of K increased in the shoot tissues of mycorrhizal plants with increasing levels of Zn. Experiment results prove that *S. setigera* is associated with the AM fungus *Rhizophagus fasciculatus*, which increases the potential to survive and grow under a moderately Zn-contaminated soil system.
Conclusion and Application of results: symbiotic associations between AMF and tropical gum trees showed a promise for successful reforestation processes in areas contaminated by heavy metals.

Keywords: Arbuscular mycorrhiza, *Sterculia setigera*, Zinc, Soil, heavy metal

INTRODUCTION

The effects of heavy metal contamination on plants result in growth inhibition and a decline in physiological and biochemical activities (Yaashikaa *et al*., 2022). Some heavy metals such as Fe, Zn, Cu, and Mn, are required for the normal growth and development of plants (Kosakivska *et al*., 2021; Arif *et al*., 2016). Zinc (Zn) has, in addition to their catalytic role, a structural function in stabilizing proteins. However, when present in high concentrations, it can cause significant detrimental consequences for the cell (Palmer and Guerinot, 2009; Ghori *et al*., 2019). *Sterculia setigera* is a tropical gum plant with a wide natural distribution in the Sudano-Guinean zone of Africa (Johnson *et al*., 2005). This plant species plays an important role in socio-economic and cultural importance in West Africa (Betty *et al*., 2011). Its exudate karaya gum is mostly extracted from private parkland and forests in Senegal, which is the world's second-largest exporter after India (Benjamin and Wilshusen, 2007). Its gum production is important for providing a source of income for many indigent smallholders (Touré *et al*., 2009). *Sterculia setigera* is a high-biomass tree able to associate with Arbuscular mycorrhizal fungi (AMF) symbiosis (Lô, 1996). One effective strategy to alleviate the stress caused by soil metal contamination is to inoculate with AMF (Moreira *et al*., 2015; Leal *et al*., 2016). AMF forms an efficient symbiosis with most plant species, helping in plant development and conferring greater tolerance to environmental stresses (Rajkumar *et al*., 2012; Ogar *et al*., 2015; Wang *et al*., 2017; Manga *et al*., 2022). Besides the extraction and immobilization of heavy metals by fungal tissues (González-Chávez *et al*., 2004); the benefits of the symbiotic association between AMF and plants are attributed to the greater volume of soil explored by the fungal hyphae, which provides greater water and nutrient absorption by the plant (Brundrett and Tedersoo, 2018; Fall *et al*., 2022). The present investigation aimed to evaluate whether AMF can improve the mineral nutrition of tropical gum trees under Zn-contaminated soil.

MATERIALS AND METHODS

Soil characteristic: The soil used in this study was collected at a 5-20 cm depth from the garden of the Botany. Soil physic-chemical characteristics are detailed in Table 1.

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

Mycorrhizal inoculum: Mycorrhizal inocula containing indigenous species were obtained from the Laboratory of Fungal Biotechnology (LBC) of the Department of Plant Biology (University Cheikh Anta Diop / Senegal). AMF was multiplied by using maize as host plant. The AMF inoculants used in the experiment were prepared by inoculating sandy soils with spores of *Rhizophagus fasciculatus*. An average density of around 40 spores per gram and 85% root infection.

Experimental procedure: Soils were first sterilized by autoclave at 120°C for 1h. Experiment treatments consisted of thorough soil and Zn (analytical grade) at 5 different levels: 0, 200, 400, 600, and 800 mg.kg^{-1}. Each nursery bag contained 2 kg of sandy soil collected from the garden of the Botany (Table 1). The experiment was done in a randomized block with five replicates. Two factors were studied: (a) Zn 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 occurred in the dark at 32°C for 3 days. Two seedlings of *S. setigera* were then transferred in 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 a 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 the root colonization rate. Shoots and roots were first rinsed with tap water and then with distilled water. Mycorrhizal colonization was evaluated using the 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. Zn, 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. 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 of variance analysis (ANOVA) were performed to partition the variance into the main effects and the interaction between inoculation and Zn level.

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 Zn. However, it strongly decreased with 600 mg·kg^{-1} Zn (Figure 1). Control plants (C) have not been colonized, their survival rate was found to be 45% at 600 mg.kg^{-1} Zn. Inoculated plants (T) were found to have a survival rate of 100% on Zn-contaminated soils up to 600 mg·kg^{-1} Zn (Figure 2). Nevertheless, at 800 mg·kg^{-1} Zn level, all plants died whatever the treatment.

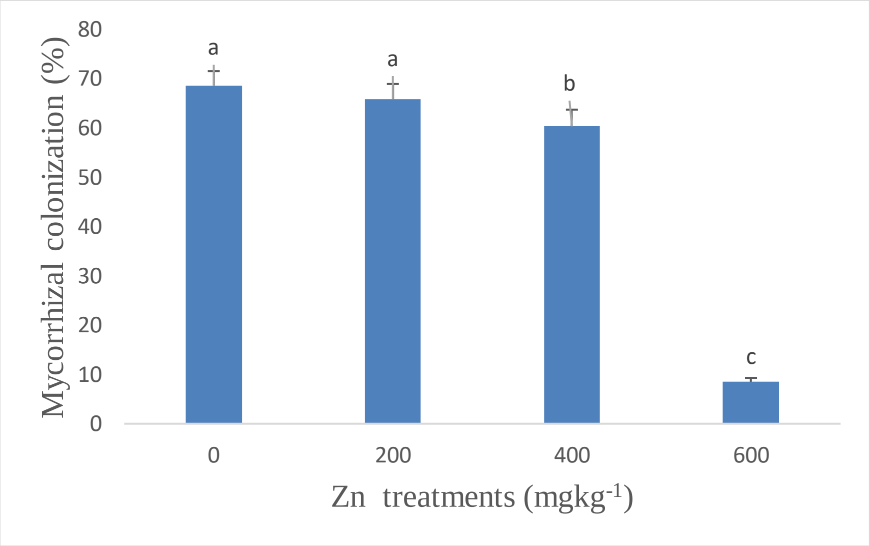

Figure 1: Arbuscular mycorrhizal fungi (AMF) colonization percentage with roots of *Sterculia setigera* plants at different Zn addition levels. Data are presented as means ± SE of the mean. Bars represent standard error. The different letters above the bars show significant differences between treatments according to the Tukey test (P<0.05).

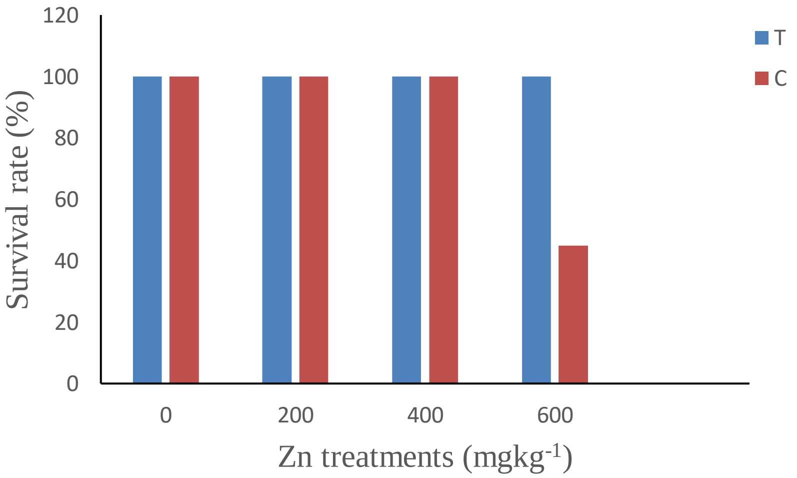

Figure 2: The survival rate of *Sterculia setigera* plants at different Zn addition levels. Non inoculated (C) and inoculated (T) with mycorrhizal fungus treatment

Mineral nutrient uptake: Nutrient concentrations in the shoots of the seedlings after harvesting are shown in Table 2. *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 Zn. No significant difference in K, Mg, Cu, and N was observed at 0 and 600 mg/kg Zn levels. However, a decrease in Ca, k, Mg, Cu, Na, Zn, N, and P was observed at 800 mg/kg Zn (Table 2). Shoot, K, Mg, Na, and Zn concentrations for Zn level conditions were significantly higher at 800 mg/kg. Analysis of variance was seen to have significantly increased Ca, K, Mg, N, and P foliar nutrient concentration by AMF inoculation. Statistical results also show that combined factors were significant for the Ca, Mg, N, and P contents. P concentration declined with increasing Zn level conditions (Table 2).

Table 2: P-values of the two-way ANOVA test for the effects of the factors "Zn 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**	**Zn**	**N**	**P**
Inoculation								
Control	**24004** ^{b}	**4545** ^{b}	**5098** ^{b}	**66** ^{a}	**2596** ^{b}	**482** ^{a}	**20** ^{b}	**373** ^{b}
AMF	**35522** ^{a}	**7095** ^{a}	**8962** ^{a}	**10** ^{a}	**4279** ^{a}	**325** ^{a}	**38** ^{a}	**615** ^{a}
Zn level (mg)								
0	**38286** ^{a}	**5375** ^{a}	**6851** ^{a}	**12** ^{b}	**3443** ^{ab}	**87** ^{b}	**40** ^{a}	**727** ^{a}
200	**21561** ^{b}	**7084** ^{a}	**6640** ^{a}	**10** ^{b}	**4021** ^{ab}	**475** ^{b}	**28** ^{a}	**670** ^{a}
400	**29745** ^{ab}	**4906** ^{a}	**7866** ^{a}	**147** ^{a}	**1763** ^{b}	**190** ^{b}	**35** ^{a}	**596** ^{a}
600	**31269** ^{ab}	**8425** ^{a}	**8189** ^{a}	**12** ^{b}	**5630** ^{a}	**1260** ^{a}	**33** ^{a}	**271** ^{b}
800	**27955** ^{ab}	**3308** ^{a}	**5605** ^{a}	**7** ^{b}	**2050** ^{b}	**85** ^{b}	**9.8** ^{b}	**205** ^{b}
P-value:								
Ino	**1.33e-07 *****	**0.0313 ***	**5.7e-12 *****	**0.173**	**0.6477**	**0.328**	**2.06e-08 *****	**1.26e-11 *****
Zn level	**0.101**	**0.4754**	**0.52957**	**0.950**	**0.0263 ***	**0.253**	**3.53e-07 *****	**< 2e-16 *****
Ino x Zn level	**8.61e-07 *****	**0.8731**	**0.00512 ****	**0.996**	**0.2693**	**0.586**	**4.64e-09 *****	**1.18e-11 *****

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

DISCUSSION

Contaminated heavy metals in the soil make vegetation establishment rather difficult (Ogar *et al.*, 2015). Mycorrhizal symbiosis is a key component in helping plants cope with adverse environmental conditions (Kumar *et al.*, 2019). Green approaches to this problem enjoy the highest public acceptance (Riaz *et al.*, 2022). The increased 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 Lô (1996). Our results show a threshold effect of AMF on plants at Zn addition levels between 400 and 600 $mg{\cdot}kg^{-1}$ Zn (Figure 1). The presence of Zn did not decrease AMF colonization. AMF was able to colonize *Sterculia* plants (8.5%) with highly contaminated soils (600 $mg.kg^{-1}$ Zn). Depressive effect of heavy metal on AMF colonization was already observed in tree seedlings transplanted to contaminated soil (Zhang *et al.*, 2012; Ferrol *et al.*, 2016). AMF has been shown to reduce plant Zn uptake in mycorrhizal plants grown under high Zn conditions (Tullio *et al.*, 2003, Watts-Williams *et al.*, 2013). These external hyphae contribute to plant uptake and immobilization of heavy metals by fungal tissues (Jin *et al.*, 2015, Bhantana *et al.*, 2021). Furthermore, other studies have reported low colonization in contaminated soils (Marinho *et al.*, 2004; Rangel *et al.*, 2014), corroborating the present results. Nevertheless, some studies showed that colonization does not decrease in plants growing with high metal contamination (Leung *et al.*, 2007; Su´arez *et al.*, 2023). Some authors showed also no correlation between root colonization and the level of metal pollution (Audet and Charest, 2006; Cicatelli *et al.*, 2010). Present results show that a high Zn level reduced the mycorrhizal colonization rate of *S. Setigera* seedlings (Figure 1). The same conclusion was also reached by Bi *et al.*, (2003) and Shen *et al.*, (2006). AMF commonly exhibits not able positive effects on plant growth by improving nutrient acquisition (Wang *et al.*, 2017), soil structure, quality, and tolerance of host plants (Zhan *et al.*, 2018). AMF structures formed barriers to bind, adsorb, and store the excessive metal ions, reduced the metal translocation from roots to shoots, and induced retention in roots (Basu *et al.*, 2021; Liu *et al.*, 2023, Wu *et al.*, 2016; Salazar *et al.*, 2018). Much research reported that AMF inoculation resulted in enhanced plant growth, and total uptake of N, P, and many other nutrients (Arriagada *et al.*, 2005; Bochicchio *et al.*, 2015; Rillig *et al.*, 2015). However, the opposite effect was observed in the Zn-contaminated soil (Watts-Williams *et al.*, 2015). The toxic effect of Zn is more important in control plants. This result suggested that AMF, *R. fasciculatus* exerts a protective effect against Zn contamination and stimulates the mineral nutrition of *S. setigera* plants even at 600 $mg.kg^{-1}$ Zn level. The effect of AMF inoculation on plant nutrition under soil Zn concentrations was different from the one observed under non-contaminated soil conditions. *S. setgigera* shoots Ca, K, Mg, Na, N, and P contents showed that the nutrient uptake was a function of the applied treatment. Colonization by *R. fasciculatus* with the addition of 200, 400, and 600 $mgKg^{-1}$ Zn improved the content of these nutrients more than the non-inoculated plants. The same results were found by Kanwal *et al.*, (2016) for inoculated plants. However, the opposite effect was observed in the Zn-contaminated soil for N and P (Dietterich *et al.*, 2017). Analyses of variance (Table 2) showed that inoculation and Zn addition levels had significant individual effects on Ca, Mg, Na, N, and P. Nevertheless, the interaction between inoculation and Zn levels was also significant for Ca, Mg, 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, N, and P more than Mg. The support of symbiotic associations between these fungi and tropical gum trees may be suggested in the application of inoculation to successfully improve mineral nutrient uptake of seedlings in the Sahelian zone agro system.

CONCLUSION AND APPLICATION OF RESUSLTS

The results suggest that mycorrhizal inoculation showed a protective effect on host plants against zinc. AMF enhances also foliar essential nutrient uptake under Zn-contaminated soil. Finally, it may be concluded that *S. setigera* with AM fungus *Rhizophagus fasciculatus* had the potential to survive and to grow under Zn moderately contaminated soil system.

COMPETING INTERESTS

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHORS’ CONTRIBUTIONS

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

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